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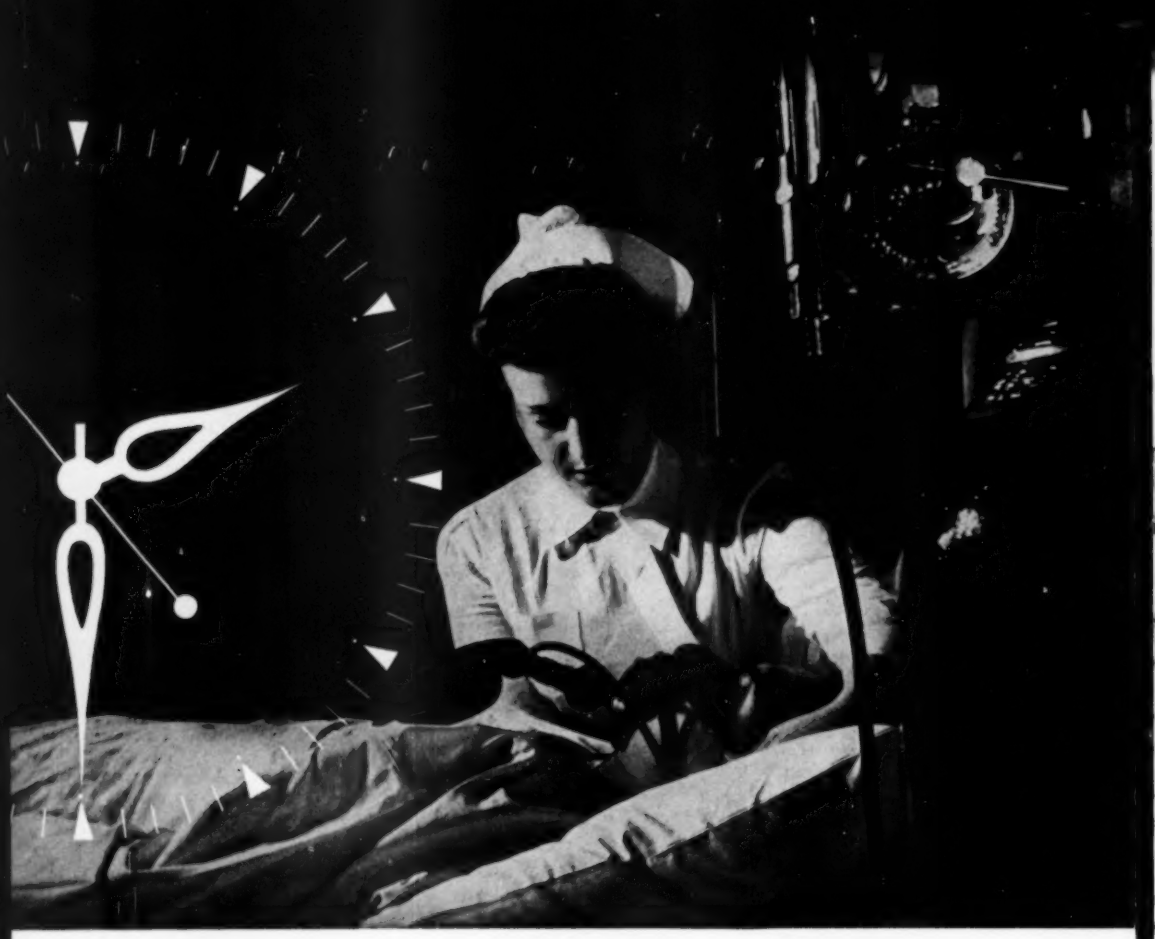

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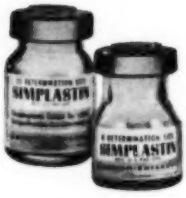

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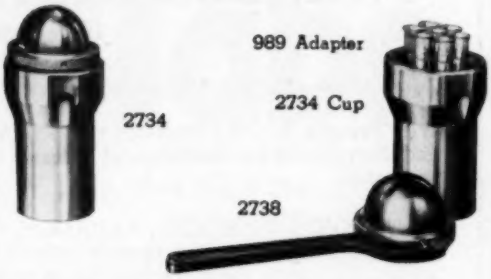

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VOLUME 57 MAY 1954 NUMBER 5

MECHANISM OF RADIATION ANENCEPHALY, ANOPHTHALMIA, AND PITUITARY ANOMALIES

Repair in the Mammalian Embryo

SAMUEL P. HICKS, M.D.
With the Assistance of Regina C. O'Brien, B.S., and Elizabeth C. Newcomb
BOSTON

THE REACTIONS to injury and mechanisms of repair and regeneration in the mammalian embryo have been little studied until recently, and the pathologic processes for abnormal development are only just beginning to be understood. Ionizing radiation has become an unusually useful tool for studying these processes not only as they relate to radiation effects in particular but to embryonic growth in general. In the rat and mouse a fairly reproducible sequence of malformative patterns can be consistently produced by irradiating females at successive periods during their gestations with single low doses of x-rays.* The nature of a given developmental abnormality is chiefly related to the time of injury to the embryo or fetus, while the degree of the abnormality is governed by the dose. In the range of 100 to 200 r in rats, for example, no measurable damage occurs to the embryo during the first eight days of gestation and normal animals result. Doses of 300 to 400 r often destroy embryos in this period, but if they survive they are apparently normal. Starting on the 9th day, coincident with the beginning of differentiation of organ systems, the sequence of malformations ensues; anencephaly or severe brain deficiencies and facial deformities result from irradiation on the 9th day; on the 10th day malformation almost exclusively limited to the eyes—anophthalmia—results; hydrocephalus and spinal anomalies occur after irradiation on the 11th day; various patterns of forebrain deficiency characterized as "microcephaly" occur from the 12th day on, diminishing toward term; cerebellar deficiencies increase toward term and extend into the newborn period. Accompanying these are a series of reproducible skeletal deformities most striking when irradiation is given in the 11- to 13-day period. Visceral abnormalities involving the heart, vessels, and urogenital system, for example, occur chiefly in the 9- to 11-day period in the rat. The terms anencephaly and anophthalmia, just as in the case of the word anemia, are not used in their absolute literal sense here because in the period mentioned deformities of the forebrain and eye range from virtual absence through a spectrum to lesser defects. Anencephaly has been applied to that malformation, resulting from irradiation on the ninth day, which in its extreme form is characterized by very little

O'Brien and Newcomb, technical research assistants.

The photographs were made by Mrs. Kathyrn H. Haley.

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* References 1-6.

forebrain, no recognizable eyes, and an extremely malformed front of the head that can scarcely be called a face (Fig. 1). Anophthalmia similarly presents itself in varying degrees, and because embryos are normally slightly unsymmetrical to start with, some "anophthalmic" animals have indeed no eye on one side and minimal development on the other. In current experiments 10-day animals have had some sort of orbit with vestiges of extraocular appendages, and so the word eye, when unqualified, will mean the structures making up the bulb.

The general reproducibility of these patterns has been confirmed by the investigators referred to above † in literally many hundreds of rats and mice, but a precise correlation of the age of the embryo at the time of irradiation with the resulting malformation has not been possible. This is because estimating the age of embryos by counting gestation days from the supposed time of coitus is at best a guess. In order to get around this time problem, a procedure has been devised which offers a much closer correlation between embryo age, time of radiation injury, and resulting malformation. For example, an animal whose duration of pregnancy has been estimated from the day of coitus (spermatozoa in the vagina) as being 11 days is

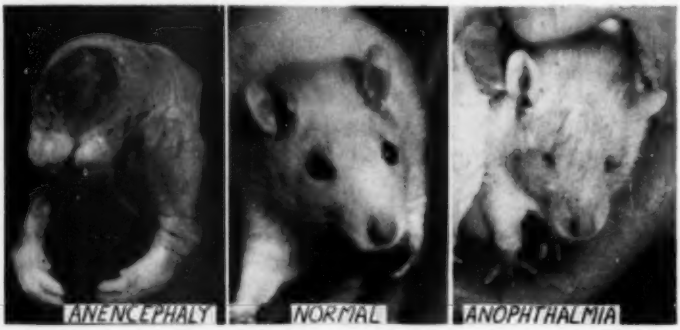

Fig. 1.—Left, anencephaly in a newborn (Caesarian) Rat, resulting from irradiation on the ninth day of gestation. Right, anophthalmia in an adult rat resulting from irradiation on the tenth day of gestation, compared with the normal. The anophthalmic animal is otherwise virtually normal.

irradiated to the whole body at 200 r. Four hours later, which is ample time for clear-cut pathologic damage from radiation to become evident,‡ embryos are operatively removed from the mother. The remaining embryos are allowed to grow to term, or they are sampled further at intervals. From this procedure a good estimate of the average age of the embryos at the time of irradiation, the sites of radiation injury as shown by damaged cells, and the malformations that result are all shown in one litter. When the litter is sampled in three or four stages the sequence of development of the malformation can be followed more closely, and in appropriate instances some of the young may be allowed to grow to adulthood for even later studies.

Prime interest in this laboratory has centered on the description of the malformations of the nervous system caused by irradiation and more particularly on the precise pathologic and chemical mechanisms by which malformations of the nervous

† References 1-6.
‡ References 1-6.

system, skeleton, and other viscera may come about. It has been demonstrated that embryonal cells are not uniformly radiosensitive but rather that considerable differences exist among them in this respect. The cells most sensitive to the destructive action of radiation are the differentiating, nonmitotic primitive neuroblasts and spongioblasts, and cells in the somites, mesenchyme and other developing organs in a probably analagous differentiating stage. In the nervous system the most primitive, actively mitotic neurectoderm is 5 to 10 times more resistant to destruction by irradiation than those cells that represent the next stage of development, namely, the primitive neuroblasts and spongioblasts. This radiosensitive stage is associated with great activity of nucleic acid and protein synthesis, and such cells have been shown to be vulnerable to a number of chemical agents that interfere with nucleic acid metabolism, and sulfhydryl enzymes apparently associated with differential growth.[7] As these differentiating cells mature into neurons and glia they become radioresistant and exhibit other coincident metabolic changes. The malformations of the nervous system that result from radiation have been shown to be initiated by knocking out the radiosensitive building blocks, and "critical periods" for certain malformations have been correlated with the shifting emphasis of this differentiating cell growth in different parts of the neuraxis during development. For example, on the 12th day a large part of the dorsal aspects of the cerebral mantles is at the primitive neuroblast and spongioblast, or radiosensitive, stage, and severe cerebral cortical defects and porencephaly result. Later, as another example, just after birth the cerebellum is most vulnerable because much of its cortex is composed of cells in the radiosensitive stage, and rats irradiated after birth grow up with specific patterns of cerebellar malformations.

What has been especially puzzling in numerous past experiments has been how on the 9th day anencephaly could result, hydrocephalus, midbrain and skeletal deformitis could occur on the 11th day, yet on the 10th day in between, anophthalmia was almost the only defect (Fig. 1). The current type of experiment in which two or more samplings are made of each litter irradiated in utero has revealed that at certain periods the mammalian embryo has a tremendous capacity to recover from injury, a property heretofore associated only with amphibians and other forms lower on the phylogenetic scale. For example, on the 10th day a four somite embryo sustains widespread necrosis of differentiating cells, yet within a day or two it has largely reconstituted itself and a virtually normal animal with anophthalmia results.

It is the threefold purpose of this report to demonstrate this reparative process in the embryo rat, to show its extremely important role in the modification of patterns of malformation resulting from irradiation at precisely known times in the 9th, 10th, and 11th day period, and to relate these findings to past experiments.

MATERIALS AND METHODS

Nine pregnant rats § were irradiated ‖ on the presumed 9th, 10th, or 11th day of gestation and their litters always sampled about four hours after irradiation and again at varying intervals later. In four instances the litters were sampled four times—including such intervals as 4, 24,

§ A Wistar strain inbred at the Harvard University Biological Laboratory for about 20 years.

‖ Radiation factors: General Electric Maximar Therapy unit, total body, 200 kv., 10 ma., 75 cm., 3 mm. Al filter approximately 70 r per minute measured in air by a Victoreen r meter and corrected for backscatter. The figures given are the literal readings obtained in each experiment.

48, 72, and 96 hours, 5, 6, and 7 days after irradiation. Because the viability of newborn animals irradiated in this 9- to 11-day period is usually poor, none of this group was allowed to go to term. Additional material consisted of a number of litters from mothers irradiated from the 8th to 13th days. These litters were examined all at once at greatly varying intervals, from two hours after irradiation through gestation, the neonatal period, or into adulthood. One litter from a mother irradiated on the 10th day was allowed to go to term, two young being studied at birth, another at one week, and four allowed to grow to adulthood. These four are the first animals successfully reared that were irradiated in the 9- to 11-day-period under study. They are apparently well developed, except for varying degrees of anophthalmia ranging from virtually no eye at all with only a trace of eyelids and appendages to some distorted formation of lids, lens, iris, choroid, retina, and optic nerve. One of these animals is used as an example in Figure 1. Judging from their litter-mates and other similar litters already studied, their brains are well-developed except for the optic tracts and nerves, which are absent or deficient. The significance of this group will be seen when later it is shown how much damage these animals sustained as embryos, yet from which they largely recovered.

Additional background material essential to the interpretation of these current experiments is formed by a large number of animals irradiated in utero from which previous timetables ¶ of radiation malformations have been constructed and from which the pathologic mechanisms responsible for them have been evolved. Frequent comparisons were made with the published data of Russell and Wilson.#

The methods of study for embryos and fetuses have been essentially serial histologic sections. The pregnant animal, at appropriate intervals after irradiation, was subjected under ether anesthesia to surgical removal of a segment or one whole horn of the uterus. The removed segment was opened, and the embryos or fetuses were fixed, usually in Bouin's fluid in situ, or, depending on their size, some dissection of the placenta was done to insure good penetration of the fixative. Embedding was in paraffin; staining was with hematoxylin and eosin, or, in some instances, Bodian's protargol, or thionine and eosin. In the case of larger fetuses, alternate ribbons of serial sections, or simple multiple rather than serial sections, were stained with hematoxylin and eosin, and additional in-between sections studied when necessary.

Since a primary aim of this study was to age embryos and fetuses as accurately as possible, this age was designated largely in terms of somites. Other characteristics, such as the degree of development of the neural folds, the heart, and other structures, was also used, especially when the embryo was cut obliquely or because it was in the stage of twisting on its long axis. Comparisons were made with our own control material, consisting of embryos and fetuses of many ages, the Harvard Embryologic Collection made available by Prof. G. B. Wislocki, and the illustrations in the classic studies of Adelmann, Butcher, Burlingame and Long.*

A large body of evidence † indicates that the pathologic effects of radiation are essentially due to action directly on the embryonal tissues. The radiosensitive cells are also vulnerable to certain chemical agents that are believed to mimic the chemical changes induced by ionizing radiations. Maternal and placental factors are excluded as important factors because the same corresponding effects occur in newborn animals or in embryos exteriorized and irradiated while the mother is shielded (Wilson ‡). The multiple operations with ether anesthesia and the selection of embryos from proximal or distal parts of the uterine horns have not measurably affected the results, and normal discrepancies in ages of embryos in a given litter have been minimal in this series.

In analyzing the data, emphasis has been laid on the early nervous system, eye, and skeleton. Gross dissections and gross stained skeletal mounts were not carried out in the present series, hence subtle cardiovascular, urogenital, and some skeletal and nasal configurations may not be evident until reconstructions are carried out.

¶ References 1-6.

References 3-5.

* References 8-10.

† References 1-7.

‡ References 4 and 5.

RESULTS

The high points of the results are as follows (Figs. 1 to 4): Severe malformations (anencephaly) of the forebrain, head, pituitary, and face with no eye formation result from irradiation when the neural plate is just being formed anteriorly and just prior to the formation of somites. Somewhat less severe malformation of the forebrain and head with very poor eye formation or anophthalmia and severe facial anomalies and irregular pituitary result from irradiation at the stage of one or two somites. From about four to seven somites, despite extensive widespread destruction of differentiating cells in the neural folds, brainstem, cord, somites, and mesenchyme, the deformity that results is essentially limited to the eye and its associated structures, and the pituitary may form slightly irregularly. In all these eye defects some sort of orbit forms, ranging from a depression in the skin with a few streaks of extraocular muscle, and an orbital cavity of sorts, to better-formed muscle and some ingrowth of cranial nerves. When retina and lens are absent, there may still be abortive formation of lids and gland appendages, and to some extent the degree of lens and retina formation is paralleled by formation of these other eye adnexa. The orbital cavity in the skull was small or proportioned to the degree of anophthalmia, as judged by serial sections and comparisons with previous gross material prepared for skeletal studies. In the 8 to 10 somite stage, the eye is still deformed, the pituitary may be normal, but dorsal enlargement of the interventricular foramen at its junction with the third ventricle may now appear. A mild deformity of the spine corresponding to damage in the region of the tail bud and also an abnormality of the cervical cord may be seen as the 14 somite stage is approached and the dorsal third ventricle deformity increases. At this stage, too, the initial damage to much of the embryo has been enormous, yet most of the animal recovers. This malformative pattern involving the dorsal ventricular region just presages a more full-blown picture with hydrocephalus and midbrain deformity with vertebral defects, as judged by several previous litters from animals irradiated on the 11th day. The results in detail follow and they will be presented by the "day of gestation" in order to demonstrate the limitations of correlation between estimated and actual embryo ages. Some histologic details will be added in the discussion. The eye deformities will be given very briefly. They embrace every possible combination of retina, iris, optic nerve, lens, orbit, and appendage deformity, and so diagnostic terms will be little used.

Results in Detail.—Ninth Day: Four rats were irradiated on the so-called ninth day after evidence of insemination. The first, K-135, (Fig. 2) received 200 r total body radiation and two embryos were removed four hours later. These have nearly flat, wide V-shaped early neural folds on frontal section, but the beginnings of the first somite cannot be discerned. Necrosis of numerous cells is evident in the neural folds and underlying mesoderm including what by location is the notochord region, but the rest of the embryo "body," amnion, primitive streak, and lateral plate tissue is intact. Desquamated dead cells are abundant in the amniotic sac in some sections. Mitotic activity is evident in the neurectoderm and in the mesenchyme, the dead cells being mostly beneath the surface of the plate and in the mesenchyme. These embryos would be between 9¼ and 9¾ days old by criteria of Butcher,[9] Adelmann,[8] and examples in the Harvard Embryo Collection. Five days later two more fetuses were removed. (Four additional involuted implantation sites were evident.) These

fetuses showed severe deficiencies in the development of the folds of the face, the forebrain and eyes—almost as severe as that in Figure 1 taken from previous experiments. The only hint of optic material is a slight resemblance of a zone of cells in part of the anterior "forebrain" to developing retina so that these animals are truly anophthalmic. The upper facial folds are underdeveloped, open, and distorted, although the tongue and lower jaw are just recognizable. Small ectopias and outward extensions of the forebrain vesicle occur in close proximity to the surface epithelium of the head. The deformity lessens considerably as the midbrain and

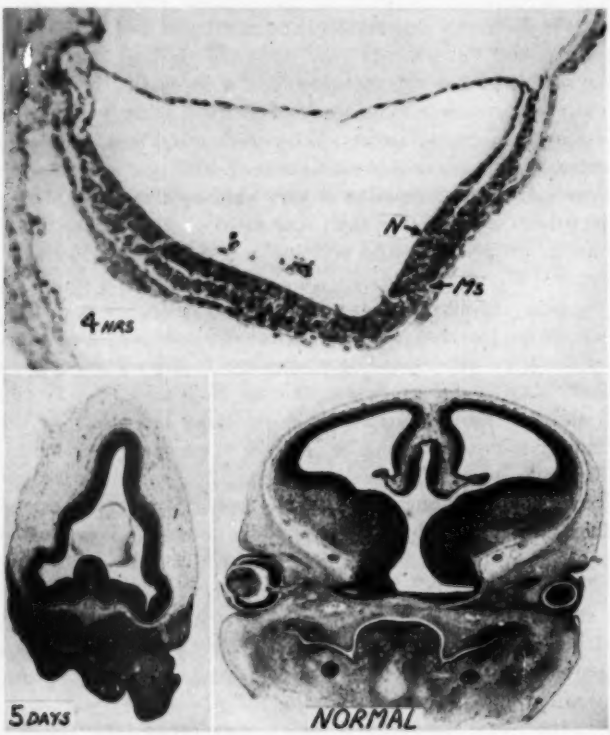

Fig. 2.—K-135, two stages in the development of anencephaly. Above, necrosis in the anterior neural plate and mesenchyme of a presomite rat embryo, four hours after irradiation (frontal section; × 200). Below, the head of a littermate five days later showing severe facial and forebrain deficiency, and lack of eyes (frontal section, low power). Lower right shows how a corresponding section of a normal head should look.

medulla are approached, and the lower medulla and cord are unremarkable. The anterior pituitary recognizably is forming irregularly. No olfactory nerves are present, and the nasal cavities are absent. The third and fourth nerves are evident as small bundles running toward what would be the eye regions. The fifth and sixth nerves are proximally somewhat developed, but their endings are indistinct in the malformed face. The internal ear is developing normally. The trigeminal ganglion itself is unremarkable, but, as noted, its branches are indistinct. So far as can be determined, a quantitative reduction in the size or number of elements in these lower

cranial nerves is not present. The still lower cranial nerves seem to be normal. The cord, spine, and remainder of the body appears normal. Cardiac anomalies are not recognized but reconstructions into three dimensional models or gross dissections were not carried out.

The second in the nine-day group, K-137, received 205 r and three embryos were removed 3½ hours later. These are slightly older than the previous presomite stage. Necrosis is essentially as in K-135. Four more members of the litter were removed six days later. They have short, unclosed eyeless faces with small, distorted cavities representing the forebrains. Development of the head and brain is scarcely better than the "anencephalics" of the first litter described. Ectopias are present including the midbrain, and distortions characterized by rosettes § of primitive neuroblasts and spongioblasts with a core of neurectoderm have developed in parts of the forebrain and anterior midbrain. The remainder of the animals' bodies is unremarkable so far as can be determined by the serial section method without reconstructions.

The third animal, K-144, (Fig. 3) received 150 r and three embryos were removed 4½ hours later, three 24 hours later, and three at 72 hours. At 4½ hours the neural folds are a little raised and the first somite is developing. Necrosis of the cells in the anterior neural folds and mesoderm is severe and conspicuous as before but still limited to this forward part of the embryo. At 24 hours a few residual necrotic cells float in the amniotic sac in one embryo that has proceeded to cover up the damage so that it is difficult to demonstrate that the anteriormost neural folds, optic pits, midbrain and facial folds are not developing correctly. The anterior neural folds have not probably begun to approach each other in closure but this is difficult to estimate. There is no phagocytosis in these ninth day irradiation animals, a reaction to injury not possible until about the 14th day. Another embryo is the same, but the third has developed into a hollow spherical mass of cells seemingly like early primitive streak material. At 72 hours the fetuses, now about 12 days old, show a forebrain similar in pattern to K-135 and K-137 but grossly less deficient. In one, the anteriormost forebrain has formed a large ectopic fold, and in the other only smaller ectopias are present. The optic stalks are present, but the developing retinas and lenses are small even at this stage. It is too early to evaluate the snout and general head shape, although they are not grossly misshapen. Pituitaries are similarly hard to evaluate, but the developing pouch of Rathke is abnormally wide in one animal.

The fourth rat, pregnant nine days, K-146, received 150 r and young were removed 4½, 48, 96 hours and 7 days after irradiation. The two 4½ hour embryos have well-raised neural folds and the first two somites are forming. Necrosis is essentially as before in its severity and distribution. At 48 hours no residual necrotic cells are present, but the face, tongue, and forebrain are smaller than normal as judged by comparable normal animals of the same somite age. The distance from the forming pituitary to the tip of the snout, for example, is short. Optic nerves and pituitary are developing, but clear-cut abnormalities cannot yet be shown. A second embryo appears to be a hollow sphere of cells (similar to primitive streak material) and is failing to develop as an embryo. At 96 hours two fetuses show a somewhat more than normally pointed snout, slightly smaller than normal forebrain and

§ References 1-6.

cerebral vesicles. The pituitary developing from Rathke's pouch shows more than the usual number of folds in its anteroposterior extent in one animal but no evidence of deformity in the other. The lens, retina, and optic nerve are smaller than normal on each side in one animal, but this cannot be certainly shown in the other. At seven days the two remaining fetuses have defects of eyelids, lenses, pars iridica, retinas, and optic nerves. In one there are no retinal structures (including pars

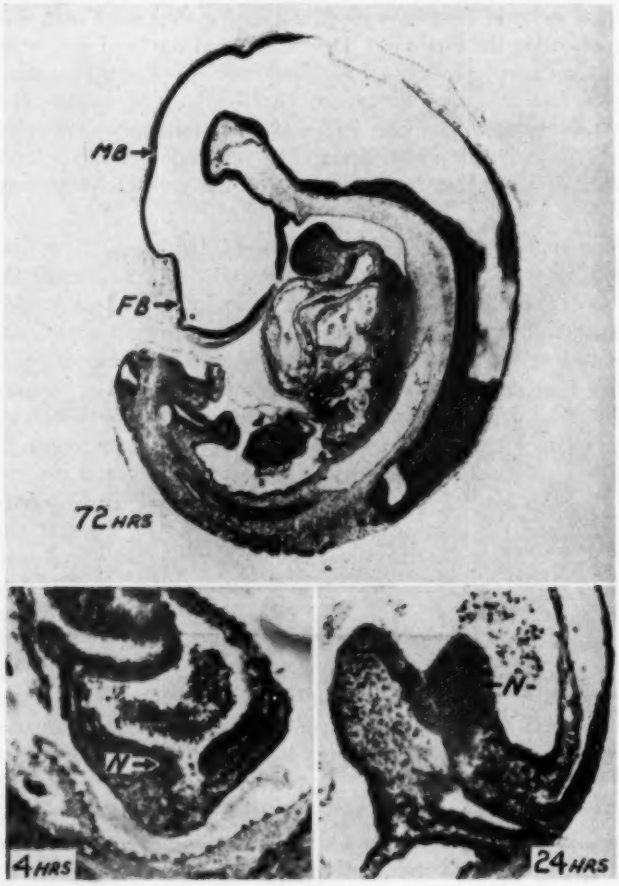

Fig. 3.—K-144, three littermates at successive stages in the development of moderate forebrain deficiency and anophthalmia. At four hours severe necrosis of neural folds (*N*) in a one somite embryo with desquamation of dead cells (frontal section; × 200). Marked recovery 24 hours later with few residual necrotic cells in amniotic cavity above (slightly oblique frontal section; × 150). Above, small irregular forebrain (*FB*) and very poor eye formation (not shown), with remainder of neuraxis and body essentially normal (low power, sagittal section).

iridica) or optic nerve on the right, but they are partly developed on the left. This same animal shows the right maxillomandibular fissure to be incompletely fused and the pituitary to be forming irregularly, in that it sends a filament through the cartilaginous base of the skull to the pharyngeal mucosa. In the second animal the

facial folds are normally closed, but the right retina and distal end of the optic nerve are absent and the lens, though present, is small and irregular. The other cranial nerves seem to be normal as is the remainder of the body in each.

Tenth Day: Two animals were irradiated on the 10th day of gestation. One, K-136, received 170 r, and three embryos were removed 4½ hours later but only one was satisfactorily sectioned. This is in the seventh somite stage. Necrosis of primitive neuroblasts and spongioblasts is marked in the neural folds, midbrain, medulla, and cord. The optic pits are forming and necrosis is marked in them and in the adjacent compact mesenchyme laterally. Necrosis seems to be just as severe, however, in the neural folds and medulla; yet as will be seen these will recover and grow into virtually normal structures. Cells in both core and medial parts of the dermatomes of the somites cephalad are necrotic but this destruction disappears as the posterior somites are approached. Four fetuses were examined five days later. One had just recently died but is generally comparable to the other three. One of these has a rudimentary left eye with a tiny optic nerve, but no retina, nerve, or lens on the right, and only a suggestion of eyelids. The pituitary is equivocally irregular. Another animal has irregular retinas and optic nerves, but no lens on the right, and its pituitary dips a little into the cartilage base below it. The fourth animal has both eyes virtually absent; there are no retinas or optic nerves, but on one side an abortive small lens has formed. The pituitary is equivocally irregular. As noted earlier, these anophthalmic and microphthalmic animals have orbits, some muscle and nerve filaments, and depression of the skin where lids should have formed, or where they have abortively formed. In these animals other abnormalities than those mentioned are absent.

The second animal, K-142, irradiated on the 10th day received 200 r and offspring were sampled at 4, 48, and 96 hours, and 7 days. Two embryos each of 10 somites were removed at four hours and they show severe necrosis in the neural folds and mesenchyme of the forebrain, head, and face regions, the brainstem and somites, as in K-136, and in the mesenchyme ventral and ventrolateral (sclerotome region) to the cord. At 48 hours two fetuses show virtually no residual necrotic cells—the volume change in the animals is enough to account for the apparent dilution of these disintegrated elements, for phagocytosis does not occur this early in development. In the first animal one eye is completely absent (that is, retina, lens, and optic nerve) while on the other side there is an optic stalk (nerve) but no retina, lens, or developing lids beyond a slight depressed irregularity of the skin where an eye should be. What may be called an orbit with the usual few appurtenances noted above is present. The second animal has both eyes, small but fairly well-formed for this stage. In both animals the pituitary is forming with more irregularity than can be construed as normal. At 96 hours one fetus was removed, and it has no left eye. The right eye is represented by an optic nerve only that tapers to nothing as it enters the orbit where the retina should be. There is a slight irregularity at the junction of the cerebral vesicle with the interventricular foramen and dorsal third ventricle, on one side. Seven days after irradiation one fetus was removed. It has small warped lenses and retinas, including the developing pars iridica region. The lids are small but otherwise unremarkable. There is still a filament of pituitary passing through a thin defect in the cartilaginous skull base to the

roof of the mouth. There is just a little dorsal outpocketing of the lateral aspect of the third ventricle just cephalad to the epithalamus. The animals' tails seem to be unremarkable.

Eleventh Day: On the 11th day of gestation three rats received radiation, and their litters were examined twice in one case and four times in each of the other two. One, K-139, was given 200 r, and two embryos were removed at four hours. These are eight somite embryos. Necrosis of differentiating cells is intense throughout the neuraxis, in the usual parts of the somites, and scattered throughout the mesenchyme. Damage to the eye region and face is marked. Mesenchymal necrosis is the same as in other embryos of similar age. The limb buds, heart structures, and tongue are spared. Desquamated dead cells are abundant in the neural vesicles and spinal cord canal. Two fetuses were removed on the 15th day of gestation. The retinas and lenses are somewhat small and irregularly formed. The remainder of the brain and spine appear normal, except that perhaps the aqueduct seems large. The pituitary seems to have developed normally.

A second rat, K-141, 11 days pregnant, received 204 r, and one eight to nine somite embryo was removed four hours later. Damage is the same as previously described in K-139. Forty-eight hours later two fetuses were removed. There is irregularity of the inner surface of the dorsal forebrain vesicle and aqueduct in one, the developing eyes appear small, but the pituitary and spine seem to be normally developing. Eyes and pituitary at this early stage, as noted before, are difficult to evaluate as to minor abnormalities. The aqueduct appears possibly too wide in the second fetus, otherwise it resembles the first. In both, the lowermost spinal cord, at the level of the region of the tail-to-be and tail gut, shows slight irregular growth with a tendency in one to form classic malformative rosettes.‖ However, it is well to recall that at this stage the tail bud and what is becoming the cervicothoracic junction are now close to each other, but future growth of much of the spine will develop between them. Two more fetuses were removed 96 hours after irradiation. The pituitary has a thin filamentous connection with the roof of the mouth posteriorly. The eyes are only slightly deformed, that is, the retina is a little irregular and the lens warped. Seven days after irradiation, two more fetuses were removed. One has fairly well-developed eyes, though smaller than normal, and a slight pituitary filament to the mouth. Another has similar malformations, and there is a dorsal enlargement of the roof of the third ventricle just posterior to the interventricular foramen and anterior to the epithalamus and aqueduct. There is otherwise no measurable reduction in the size of the posterior cerebral mantle here.

The third animal, K-143 (Fig. 4), irradiated on the 11th day received 200 r, and two embryos were removed four hours later. These have 14 somites, and necrosis is extensive and severe in the usual radiosensitive differentiating cells throughout the neuraxis. Necrosis in the somites involves the outer dermatome cortex, especially laterally and dorsally, and the core and sclerotome cells. The mitotic layer, as seen in some sections between the cortex and myotome (which is now beginning to look like bundles of very young muscle) are spared. Necrosis is conspicuous in what are now beginning to be demarcated future vertebral bodies. Segmental vessels from the aorta are undamaged, and the notochord is apparently intact. Three fetuses were removed 48 hours later. There are rare residual necrotic

‖ References 1-6.

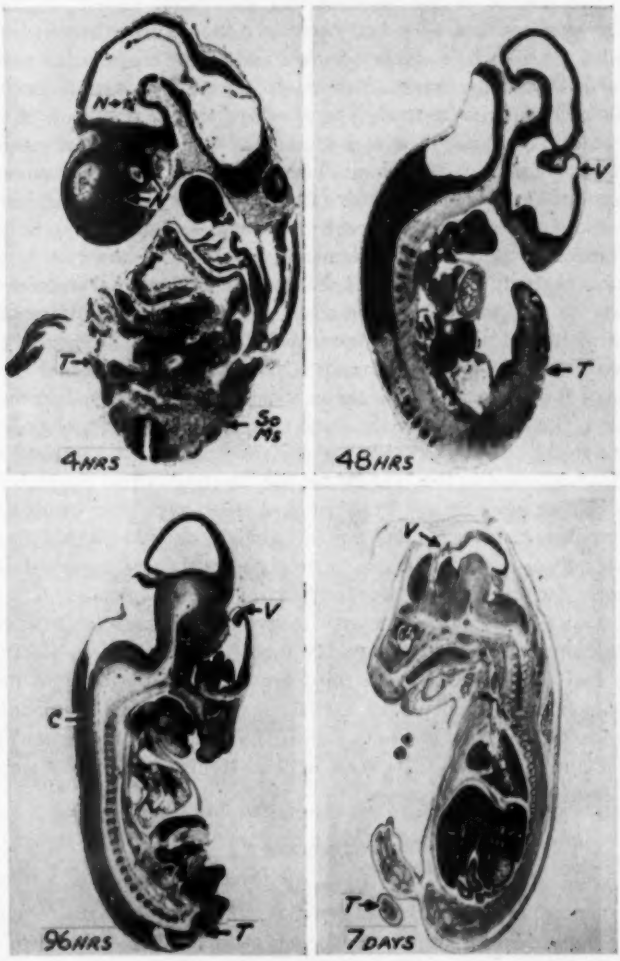

Fig. 4.—K-143, four littermates at successive stages of development of cerebral ventricular deformity (*V*), eye deficiencies, mild cervical cord and tail deformities. The animal at four hours is 14 somites and about 5 mm. long, the one at seven days is nearly 2 cm. long. At four hours there is severe necrosis of primitive differentiating nonmitotic cells throughout the embryo, as shown by fine stippling at this low magnification. Some significant places in the neuraxis and optic stalk (*N*) with desquamated dead cells in the neural canal, somites (*SO*), mesenchyme (*MS*), and tail bud (*T*) are noted. At the succeeding intervals the tail and dorsal cerebral ventricular region become malformed and at 96 hours a minor irregularity of the cord (*C*) is shown.

cells in the brain stem and cord, but generally good development is proceeding, despite the tremendous numbers of necrotic cells seen in the littermates just described. The somites in the proximal tail and sacral region are lobulated and irregular in dorsal outline. Residual necrotic cells are abundant in some sections along the shell of the whole cerebral vesicle and in the medulla but not within the parenchyma itself to any extent. Posteriorly the cerebral vesicle is thin and not pushing back as much as normal. The eyes are small. The floor of the medulla (fourth ventricle) is focally irregular in contour at its bend. The pituitary cannot be seen to be irregular at this stage. Two more fetuses were removed 96 hours after irradiation. These have a number of abnormalities easily recognized. The eyes are small with distorted lenses, although the retinas and developing uveas are well-formed. There is a thin pituitary filament to the roof of the mouth as described in the other series, and in one animal the floor of the hypothalamus is not quite in contact with the pituitary. There is a small projection of the surface of the center of the floor of the fourth ventricle upward at the angle of its bend. Both animals have kinked stubby tails with deformation of the spinal cord canal corresponding to the base of the tail, and in one the cervical cord is slightly deformed centrally and ventrally. The dorsal part of the third ventricle forms bilateral pockets upward just anterior to the epithalamus, and the posterior parts of the cerebral vesicles are not as thick or as large as they should be, nor do they extend posteriorly as far as they should. Three more fetuses were removed seven days after irradiation. Grossly they have crooked stubby tails and one has a slight outpocketing of the scalp with local edema, but microscopically this is simply a focal loose region of skin, not an encephalocoele (which is more characteristic of 12-day animals). The lenses and retinas are somewhat malformed except in one animal one of whose eyes is very severely underdeveloped, there being little more than an orbit as described earlier. There is dorsal outpocketing of the third ventricle just posterior to the interventricular foramen and anterior to the epithalamus, as in the other animals described, very marked in two animals, less so in a third. The surface of the center of the fourth ventricle is slightly puckered at its bend, but this seems to be less consequential than it was in littermates at earlier stages.

COMMENT

Examination of members of the same litter at successive intervals during gestation, starting four hours after irradiation, makes it possible now to take much of the guesswork out of correlating malformations with the age of the embryo at the time of injury. The present experiments establish that the anencephalic pattern characterizes the presomite to one or two somite stage and its severest form occurs a little earlier in development (presomite stage) than previous, less accurately timed, experiments had suggested. The two to eight somite stage was characterized chiefly and almost exclusively by degrees of anophthalmia, but as 14 somites was approached, spinal cord, tail, and third ventricle deformities began to appear.

What stands out is the large discrepancy between the enormous destruction of differentiating cells in these early embryos and the resultant very slight malformation that occurs in some stages. This regenerative capacity of the mammalian embryo in the face of injury makes it necessary to revise our previous concept of

relating the mechanism of malformations almost entirely to destruction of building blocks. Injury has, of course, been related to malformation but repair was an unknown factor.¶

Since these sites of injury and the resultant malformations are now known, the parts that cannot repair themselves, such as the optic pits, need to be scrutinized to see why they behave differently. Repair in the nervous system comes from the mitotic primitive germinal neuroepithelium (neurectoderm) and in the somites from corresponding primitive dividing cells. In the looser mesenchyme this relation between most primitive dividing cells and their differentiating derivatives is harder to establish, but radiation damage seems here to affect zones of condensation that precede structural differentiation. In the neural tube, early cerebral vesicles, and optic pits the neurectoderm lines the cavity and the radiosensitive spongioblasts and neuroblasts bud off, as it were, from this source, forming a layer whose thickness varies from a few cells to a thick zone, depending on the situation and age of the embryo. Nearly the same relation obtains in the early neural plate and neural folds, where the mitotic cells are on the surface and growth in numbers is lateral and downward. There are some things in common among these neurogenous zones that give rise to malformations after irradiation but cannot recover like adjacent regions. The optic pits, the neural part of the hypophysis, and the developing cerebral vesicle all are places where there is not only differentiating cellular growth but also differentiation of an outward growing pocket, a new structural unit. A distinction may then be made between proliferation of differentiating cells with an accumulation of these cells locally, and proliferation of differentiating cells to define and build a new structure, such as an optic pit or a tail bud or a cerebral vesicle. It is these differentiating structures that get into trouble.

Is there any visible difference between these zones that cannot recover from injury and those that can, aside from this structural differentiation just mentioned? Cytologically there does not seem to be any measurable difference in the mitotic rate of the neurectoderm from which optic pits and cerebral vesicles differentiate. The rate of growth and differentiation of the primitive neuroblasts that spring from the optic pit neurectoderm to form an optic nerve and a retina is, of course, somehow different from the adjacent neural fold that will later form the cerebral vesicle, but the differences are at least not presently apparent in routine sections. There does seem to be a condensation of mesenchymal cells adjacent to the optic pits and the same relation is suggested at the floor of the neural groove where the hypophyseal region is to appear. Similar condensation is associated with the expanding neural tube as it finds itself adjacent to the tail bud. The finding is not prominent adjacent to the developing posterior aspect of the cerebral vesicle, but embryos older than 14 somites may add information on this. It is notable, however, that these mesenchymal condensations are especially radiosensitive and seem, therefore, to be analagous to differentiating mesenchyme seen in other situations such as in the somites or later in the forming vertebral bodies (sclerotomes), where radionecrosis is notable. Further data are needed to establish whether damage to the differentiating mesenchyme is the important factor in

¶ References 1-6.

determining these neural malformations. Histochemical stains may reveal differences between vulnerable and nonvulnerable zones and their adjacent mesenchyme that ordinary stains do not. Theoretical considerations of organizers and inductors at this stage of the experiments is best postponed, and it is interesting to note in this connection that in some animals with deficient eyes there was a lens and other appendages with no retina, and in another a retina and optic nerve with no trace of lens.

The coincidence of tail and upper spinal cord anomalies presents a paradox at first glance. At about the time of the 11th somite # the tail bud is becoming prominent, and the distal end of the neural tube is in a phase of rapid extension, and they are adjacent. Both are structurally differentiating and vulnerable to malformation for whatever reasons such zones are vulnerable. Subsequent growth of the thoracic and lumbar body regions comes from the undifferentiated mesenchyme of the "cervical cord-tail bud" junction, and the two structures are subsequently widely separated. Hence there is the paradox of coincident cervical and tail anomalies. From past and present experiments we can now anticipate approximately that as the 14 to 30 somite stage (11th to 12th day) is explored the cord malformations will progress downward, the tail malformations will decrease, vertebral irregularities will increase, and developing limbs will show deformities.

In previous experiments in this and other laboratories * a given malformation sometimes seemed to be spread over a broader time period during development than could reasonably be expected. For example, eye, foot, and tail deformities seemed to occur at times when it was believed, on a basis of estimated gestation dates, that the anlagen for the affected parts were either not yet present or had passed the vulnerable stage. Also a large dose seemed to increase this spread and a smaller dose narrowed it. There seems now to be a satisfactory explanation for these seeming discrepancies. The time for a specific deformity was based on a number of litters whose gestational time was at best an estimate, so that the results were an average of a number of litters whose stage of development was only approximately similar. Thus the optic pits have a peak radiosensitivity at, let us say, two somites because the cells vulnerable to radiation are at a peak of differential cytologic and structural growth which is presently believed to be associated with, and dependent on, marked sulfhydryl enzyme activity concerned with nucleic acid and protein synthesis.[7] Increasing the dose can begin to interrupt these processes a little earlier and continue a little later than would be the case with a median dose. Decreasing the dose would, similarly, narrow the time during which these chemical activities could be interrupted. There is, of course, a very practical limit to how precisely these peaks may be known, and this limit is determined by such factors as slight variations in age within the litter and differences between the two sides of the individual embryo. Nevertheless, the method of sampling the same litter at different times after irradiation brings us much closer to the actual processes that govern malformations than had been hitherto possible.

References 8-10.

* References 1-6.

SUMMARY

Low doses of x-rays administered to pregnant rats and mice cause in the embryos and fetuses a fairly reproducible series of malformations that have been approximately correlated with the time of injury. In the rat, starting in the 9th day, irradiation produces severe head and, especially, brain malformations (anencephaly), anophthalmia on the 10th day, hydrocephalus and other brain deformities on the 11th and 12th days, cord and spinal anomalies on the 11th to 13th days, and various patterns of microcephaly and later cerebellar deformities from the 12th day till the neonatal period. Other visceral, skeletal, and limb deformities occur in the 9 to 12 day period. No series of really precise correlations between the age of the embryo at the time of radiation injury and the resultant malformation had been available.

The present study utilizes a procedure of irradiating the pregnant animal on a certain estimated day of gestation, removing some embryos four hours later (ample time for radiation necrosis of vulnerable cells to become visible), and removing the remaining fetuses at one or more subsequent times. This method provides a much closer approximation of the average age of the litter of embryos, the sites of radiation injury, and the resultant malformation, all in one litter. It had been previously established that malformations, especially of the nervous system, were closely associated with or initiated by the destruction of the nonmitotic differentiating primitive neuroblasts and spongioblasts which are selectively killed by low doses of radiation.† This concept must now be modified by the present more precise experiments. These demonstrate that despite extensive necrosis of differentiating neural cells and cells in an analogous stage in other developing systems, complete or nearly complete repair can occur in many zones. Radiation malformations are therefore the result of a balance between radiation damage and the capacity to repair in any given anlage or developing zone. In the presomite to first somites stage (ninth day), differentiating cells in the neural plate and mesenchyme are destroyed by radiation and anencephaly and anophthalmia results. Paradoxically on the 10th day three to eight somites, malformation is essentially limited to the eyes; yet radionecrosis in the embryo is very severe in the neural folds, neural tube and groove, somites, and condensing areas of mesenchyme. Virtually only the optic pits (and their adjacent mesenchyme and ectoderm?) fail to recover. Repair is from the primitive mitotic cells, such as neurectoderm and some primitive mesenchyme cells, which are relatively radioresistant.

Some of the possible underlying reasons for the malformative patterns and future lines of investigation are also discussed.

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ASPERGILLOSIS OF LUNGS AND DUODENUM WITH FATAL INTESTINAL HEMORRHAGE

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WE WOULD like to report a case of fatal gastrointestinal hemorrhage from a duodenal ulcer secondarily involved by Aspergillus fumigatus. In this case the Aspergillus invaded and disrupted the wall of the artery of hemorrhage to such a degree that the fungus must be considered a contributory factor leading to rupture and fatal hemorrhage. We have been unable to find any previous reports of aspergillous involvement of a duodenal ulcer associated with a fatal intestinal hemorrhage.

REPORT OF A CASE

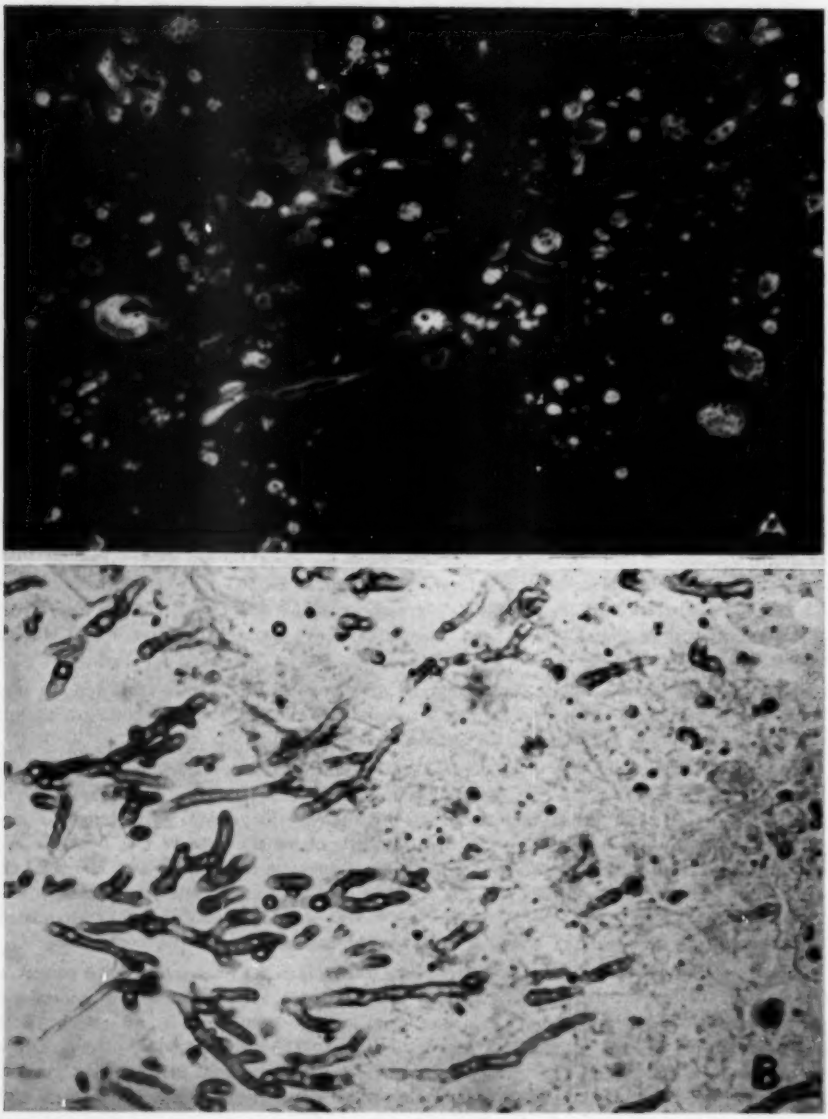
History.—This 64-year-old white seaman reported to the purser aboard ship on Dec. 12, 1951, complaining of painful hemorrhoids and fever. He was given 200,000 units of oral penicillin and 0.5 gm. of sulfadiazine three times daily. Progressive swelling of the anus and the right groin developed. Four days prior to hospitalization sloughing of the perianal tissues occurred. He was hospitalized on Dec. 21.

Physical Examination.—There were gangrene and sloughing of the anal canal and the contents of the ischiorectal fossa. Inflammatory edema involved the scrotum, both groins, and extended up the left rectus sheath to the xyphoid process.

Progress.—The patient was given 600,000 units of intramuscular penicillin daily. He was given therapeutic doses of gas gangrene and tetanus antitoxins. On Dec. 24, abscesses in the right groin and left rectus sheath were drained. Thereafter he was given oxytetracycline (Terramycin), 2 gm. daily. Postoperatively the patient showed symptomatic improvement and there was resolution of the inflammatory process. On Dec. 28, a transverse colostomy was performed. On Dec. 30, the patient complained of dysphagia and developed a severe chronic cough productive of a very thick mucoid sputum. On Jan. 2, 1952, the patient was found dead in bed approximately 20 minutes after routine ward rounds, at which time he had been alert and uncomplaining.

Autopsy Findings.—Gross Examination: There was ulceration of the scrotum and perineum. Suppuration of the subcutaneous fat extended from this area anteriorly over the left side of the abdomen. A 1 cm. in diameter fistula was found in the rectum 4 cm. from the anus. Both lungs were firmly adherent to the chest wall at the apices. The pleura of both apices contained several large calcified plaques. Sections of the lungs revealed numerous discrete, soft, grayish-white peribronchial infiltrates (Figure, *A*). There were several small calcified nodules in both apices. No cavities were present. The tracheobronchial nodes were enlarged and contained small calcified nodules. The stomach, duodenum, and jejunum contained a large cast of clotted blood. Black tarry intestinal contents were present in the lower ileum and colon. In the duodenum, 2.5 cm. from the pylorus, was a large deep ulcer 3 cm. in diameter. The base of the ulcer was firmly adherent to the pancreas. A small artery opened into the base of the ulcer.

From the Departments of Pathology and Surgery, United States Public Health Service Hospital.



A, cut surface of lung, right lower lobe; gray infiltrates around bronchioles and blood vessels; actual size. *B*, septate, branching mycelia in necrotic wall of duodenal artery; Gridley fungus stain; × 500.

Microscopic Examination: The peribronchial infiltrates were seen as small abscesses containing branching, septate mycelial elements, surrounded by a heavy infiltration of neutrophiles. The pulmonary parenchyma in these areas of suppuration was destroyed. The mycelia branched laterally and showed no evidence of spore formation. No fungi or acid-fast bacilli were seen in the calcified apical nodules or hilar lymph nodes.

The duodenal ulcer showed a severe subacute inflammation. In the base of the ulcer were numerous branching, septate mycelia identical to the fungus seen in the lungs. These mycelia extended deep into the wall of the duodenum almost to the adjacent pancreatic tissue. The fungus elements were surrounded by polymorphonuclear leucocytes. The mycelia infiltrated the entire thickness of a small artery opening into the base of the ulcer (Figure, *B*). The wall of the artery was edematous, necrotic, and infiltrated by polymorphonuclear leucocytes. The fungus extended along the arterial wall into the deeper portions of the ulcer for a distance of approximately 1 cm. In addition, a few colonies of yeast-like cells were found in the superficial necrotic layer of the ulcer. These cells were morphologically identified as a Candida species. The Candida did not infiltrate the ruptured artery. Tissues from the rectal fistula and abdominal wall did not contain fungi.

Cultures: Four cultures upon Sabouraud's media, made directly from separate infiltrations in the lung, yielded a heavy growth of an Aspergillus species identified as Aspergillus fumigatus by Dr. Chester W. Emmons at the National Institutes of Health, Bethesda, Md.

The slides were submitted to the Armed Forces Institute of Pathology (AFIP Accession No. 517207). They identified the fungus morphologically as Aspergillus in the lungs and duodenum. The small yeast-like cells were noted in the necrotic zone of the duodenal ulcer, and appeared to be a Candida species.

COMMENT

An investigation of the possible source of infection in this case revealed that the patient had served from Oct. 17, to Dec. 21, 1951, aboard a ship carrying a full cargo of grain. Since aspergillosis has been found most often in people who have had some contact with whole grain, it is possible that this patient acquired the fungus spores while aboard his ship.

Two factors contributed to the growth of the fungus: (1) general debility from the subcutaneous infection, and (2) the extensive use of antibiotics. The case of acute pulmonary aspergillosis reported by Cooper [1] developed in a patient suffering from a severe abdominal infection. Enhancement of virulence in fungus infections by the use of antibiotics is discussed thoroughly by Zimmerman.[2]

In a case of disseminated aspergillosis reported by Grekin,[3] lesions were present in the wall of the duodenum as small granulomas. No ulcerations were described. Rankin [4] reported a case of disseminated aspergillosis and moniliasis that had ulcerations of the esophagus, stomach, and duodenum. These ulcers were infiltrated predominately by Candida, however. In both cases there was evidence of hematogenous spread to many organs. In our case there was no evidence of involvement of other organs. A single hematogenous localization in the duodenum is not likely. There was no past history of peptic ulcer. In view of the subacute stage of inflammation seen, the ulcer may have developed as a result of general debility, with superimposition of the fungus via the alimentary tract.

We feel that the invasion of the duodenal artery by the Aspergillus was a significant contributory factor leading to rupture of the vessel. The ability of Aspergillus to invade and rupture arteries has been demonstrated in a previous report by McKee.[5] He showed Aspergilli in the wall of the internal carotid artery producing acute arteritis with rupture and extensive subarachnoid hemorrhage. In the case described by Rankin [4] the Aspergillus invaded the pulmonary, splenic, and renal arteries with thrombosis and infarction.

SUMMARY

A case of acute Aspergillus fumigatus infection is described in a patient suffering from severe subcutaneous inflammation secondary to a rectal fistula. Death was a result of a severe gastrointestinal hemorrhage from a duodenal ulcer actively invaded by the fungus.

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ALTERATIONS IN THE GLIAL CELLS FOLLOWING IRRADIATION OF THE BRAIN IN PRIMATES

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THE CENTRAL nervous system has been generally considered as resistant to x-irradiation, and, as a consequence thereof, very few extensive histopathological studies have been made of the effects of x-rays and other radiations on the normal central nervous system and on the tumors of the central nervous system of man. However, with the development of more precise means of delivering x-rays and other radiations, as well as the increased availability of new types of radiations suitable for study, a strong interest has developed in determining the effects of the new radiations, as well as reevaluating the effects of conventional x-rays on the normal central nervous system of primates and on the tumors of the central nervous system of man. In this respect, we have been studying, for some five years, the effects of conventional x-rays and very high-energy x-rays on the tumors of the central nervous system of man and on the normal nervous system of monkey and man. Our sources of low-energy x-rays have been the conventional x-ray machines at 200 to 400 kv., whereas our source of high-energy x-rays has been the medical betatron at the University of Illinois, which can produce x-rays at a peak intensity of 23 mev (million electron volts). The betatron can also emit high-energy electrons as a beam suitable for both therapy and investigative purposes.[7] The high-energy potential of the betatron enables one to produce beams of x-rays which are more penetrating, free from side-scatter, and more homogeneous in dose distribution than beams of x-rays of low energy. With these very useful physical features of high-energy x-rays plus the precision of radiation localization possible with the betatron, one can demonstrate that the nervous tissue is responsive to x-rays,* that the hypothalamus is peculiarly responsive to x-rays,[8] that the brain stem will not tolerate large doses of x-rays or high-energy electrons,[4] and that the normal electroencephalographic pattern of the brain of a monkey can be altered by low doses of x-rays.[5] In addition, it has been possible to determine a general histopathological pattern of response of the glioblastoma multiforme to x-irradiation by a combined study of tissues obtained from patients receiving therapy from the betatron or from conventional x-ray machines.[6]

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* References 1 and 2.

The high-energy x-rays from the betatron have been highly advantageous in this study because of the intensity of radiation effect obtainable by means of the methods of irradiation employed.

METHODS OF STUDY

Our methods of study of experimental irradiation of the central nervous system of the monkey have been given in detail in previous publications.† In our investigations on humans, the general plan has been to study the reactions of the glial cells in normal cerebral tissues which had been irradiated during the course of therapy, but which were not in the immediate vicinity of the tumor. For example, a patient who had received a course of x-ray therapy for a brain stem glioma, would have the entire cerebellum and both occipital lobes serially sectioned, in addition to the brain stem and tumor, for a study of the radiation responses. The cerebellum and occipital lobes would receive a percentage of the tumor dose during the course of therapy. In patients with cortical neoplasms, normal irradiated tissues were usually obtained from the opposite cortex for study. In all cases, an effort was made to select normal tissues which had received a readily determinable percentage of the tumor dose, and yet were sufficiently divorced anatomically from the tumor so that the glial changes were not part of any local reaction to the tumor per se. In general, in the human studies, the tissues were obtained at autopsy. More recently, tissues for study have been obtained from patients who have required additional surgery because of tumor recurrence or because evidence of delayed radionecrosis appeared in and about the tumor bed some prolonged interval after the initial course of therapy.

The tissues were usually fixed in formaldehyde; for silver impregnations small blocks of tissue were fixed in formol-ammonium bromide. The formalin-fixed tissues were imbedded in nitrocellulose and cut serially at 20 μ. Three series of sections were made, beginning with the 20th, 21st, and 22nd sections. Thereafter every consecutive 20th section was saved and the first individual series was stained with hematoxylin and eosin, the second by the method of Nissl, and the third by the method of Weil and Van Gieson. Whenever a very large portion of human cortex was required for study, every consecutive 50th, 51st, and 52nd section was saved in lieu of every 20th section to make the three series, thereby economizing considerably on the amount of labor involved and the number of sections to be studied.

OBSERVATIONS

The normal adult glial cells, i. e., the astrocytes, the microglia, and the oligodendroglia can respond to x-irradiation. The type of response as well as the manner of response can be readily correlated with (1) the total-dose of radiation, (2) the intensity of dose administration, (3) the uniformity of dose-distribution within the tissue, and (4) the duration of time of observation after irradiation. It would appear to be unlikely that the various types of ionizing or nonionizing radiations will yield different types or patterns of responses, i. e., qualitative differences, but there is ample evidence to believe that all radiations studied thus far may give quantitative differences in biological effects.

1. *Glial Responses as Related to Total Dose of Radiation.*—When a comparatively large dose (7,000 r or more) of high-energy x-rays (23 mev) is administered in a single exposure via a 1.0 or 2.5 cm. circular port transfrontally, a pattern of acute radionecrosis of the irradiated area evolves, in which all the cellular elements and nearly all the smaller vascular channels are destroyed. Many of the medium-sized, as well as the large arteries and veins, are still patent. Their endothelial cells are usually swollen and hypertrophied. As the irradiated area undergoes necrosis, very few, if any, compound granular cells or microglia appear in the irradiated area

† References 1 and 2.

or in the adjacent nonirradiated tissues. The astrocytes at first appear to swell and to proliferate somewhat. They soon fragment and disintegrate so completely that specimens at four to six weeks after irradiation are usually totally devoid of any viable glial or neuronal cells. The responses of the oligodendroglia are more difficult to follow, but in general they show an early swelling of their cytoplasm and nuclear pyknosis followed by a complete breakdown of the entire cell. The neurons are acutely necrosed by the irradiation, but surprisingly little or no neuronophagia of the disintegrating nerve cells by the glial cells occurs. The phenomenon of neuronophagia by the neuroglia is a relatively constant finding in the reactions of the central nervous system to ischemic and toxic states. It would, therefore, appear that in acute radiation damage to the brain the reactive capacities and the scavenger propensities of

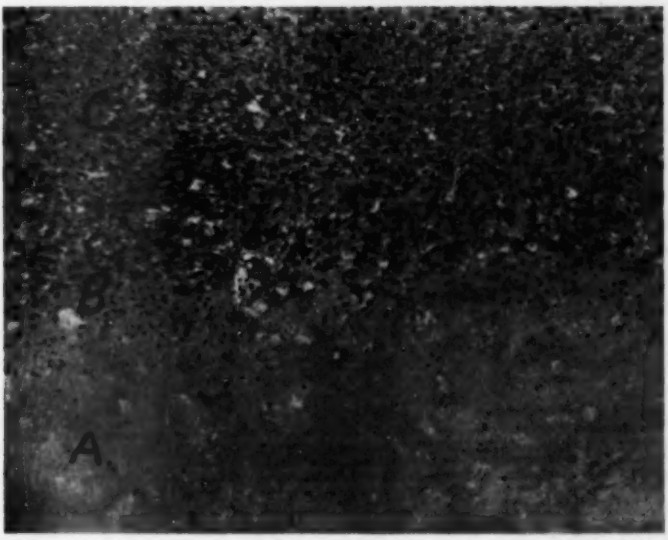

Fig. 1.—Acute radionecrosis in frontal lobe of a monkey six weeks after the administration of 7,000 r of 23 mev x-rays. Zone *A* represents the area of necrosis which is almost acellular at this time. *C* indicates the normal nonirradiated area. The junctional area *B* shows very slight glial reaction and no vascular proliferation. Hematoxylin and eosin stain; × 100.

the neuroglia are drastically affected by the radiations. Figure 1 shows the appearance of the frontal area of a monkey at six weeks following the administration of 7,000 r of 23 mev x-rays ‡ in a single exposure. Zone *A* represents the area of acute radionecrosis, which is now almost completely acellular whereas *C* indicates the normal nonirradiated area. The junctional area *B* shows very little glial proliferation at this time except for a slight increase in the number of astrocytes. However, with the passage of many months, the glial reactivity of the junctional area *B* recov-

‡ On the basis of existing data, this dose of 7,000 of 23 mev x-rays is about equivalent to 4,200 r of 200 kv. x-rays. References 1 and 2 give explanation of the decreased effectiveness of high energy radiations.

ers and a glial scar slowly develops. At a year or more after radiation the reactive gliosis and fibrosis may become very intense. At six weeks postradiation the junctional area still shows no evidence of vascular proliferation.

This impairment of the reactivity of the neuroglia by irradiation can be considered somewhat analogous to the disturbances in fibroplasia observed in other tissues following irradiation.

As the total dose of x-rays administered is reduced below the acute necrotizing dose, the reactive capacities of the neuroglia are still impaired by the radiation. In the dose-range of 3,000 to 5,000 r of 23 mev x-rays, wherein small areas of acute necrosis may appear and intense swelling of all the neuronal and glial elements, as well as of the myelin, occur, the processes of neuronophagia and gitter cell production are still inhibited by the irradiation. The astroglia appear to be much more hardy and at first increase in numbers about the areas of necrosis. This early proliferation of the astrocytes about the areas of necrosis soon subsides so that very little reactive gliosis persists about the acutely necrosed areas. There is at first an incapacity of the astroglia to gliose the necrosed areas. Months later, however, the astrocytes show a slow recovery and, with the further progression of time, i. e., a year or more after irradiation, an intense gliosis and fibrosis appears within and about the radioinjured areas. At this late period an intense hypertrophy of the individual glial cell occurs, which will be described under Section 4.

With the administration of lower dosages of 23 mev x-rays (1,500 to 3,000 r), extensive tissue edema, inflammatory cell reactions, swelling of the myelin, neurons, and endothelial cells occur, in addition to perivascular hemorrhages and exudates. The acute reactions of the glial cells are not particularly evident in this dose-range. Some of the oligodendroglia show nuclear pyknosis and cytoplasmic swelling. The acute inflammatory reaction, edema, and cellular swelling subside shortly thereafter, but after a latent interval some months later a radionecrosis of the white matter in particular occurs. The immediate glial responses to these areas of delayed or latent radionecrosis are very slight at first. Again with the passage of time, an intense reactive gliosis and fibrosis appears in and about the areas of necrosis.

In general, it would appear that the functions of the glial cells are depressed or inhibited by x-irradiation. With the passage of time a recovery of the glial elements occurs, and they perform their function with a somewhat increased and perhaps "uninhibited" vigor, thereby producing a very intense gliosis of previously radionecrosed or radiation-injured areas.

2. *Glial Responses as Related to Intensity of Dose.*—In general the effects of a specific dose of radiation on an adult tissue are greater when the dose is administered in a single exposure than when the same dose is fractionated over a period of days or weeks. It would appear from our preliminary experimental studies with high energy electron beams, that a very rapid rate of administration of a single dose will also give a greater intensity of effect than the same dose given at a much slower dose-rate. It is possible to deliver the beams of high energy electrons, as produced by the betatron, at variable dose-rates of 75 r to 1,000 r or more per minute. Our preliminary data thus far would indicate that the acute necrotizing doses, as well as the dosages required for inhibition of the glial responses, are less with a dose-rate of 1,000 r or more per minute than with dose-rates of 75 r per minute. Further studies are necessary to fully establish this difference of effect with dose-rate.

Therefore in comparing the experimental results of various investigators studying the effects of radiation on the central nervous system, the factors of dose-rate and fractionation of dose become important factors in the evaluation of the effects obtained.

3. *Glial Responses as Related to Uniformity of Dose-Distribution Within the Tissue.*—When a beam of high energy x-rays is utilized in the manner employed in our experimental studies in the monkey,§ the effects on the glial cells are equal throughout the pathway of the beam because a relatively uniform dose-distribution of radiation is obtained in the tissue. When studies are made of the effects of x-irradiation from a conventional x-ray unit or of the effects of gamma irradiation from a radon seed implant or other isotope, due consideration must be given to the lack of uniform dose-distribution in the tissue secured with such sources of radia-

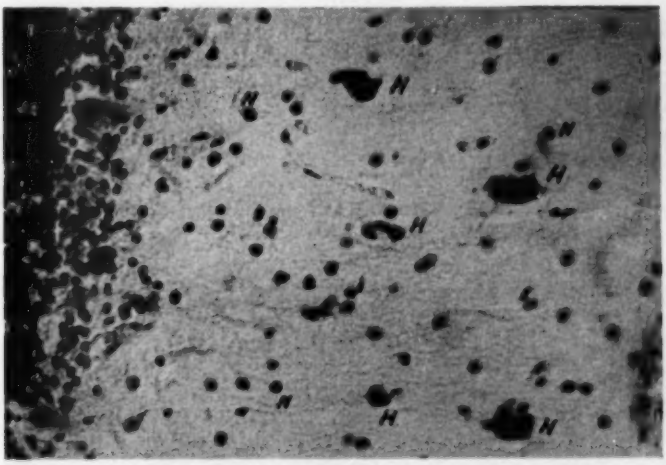

Fig. 2.—Hypertrophy of the glial cells in the molecular layer of the cerebellum of a patient who had received a course of x-ray therapy some 15 months prior to death. Internal granule cell layer and Purkinje cells on left side. Hypertrophied glial cells (*H*) in molecular layer. Normal glial cells (*N*). Weil-Van Gieson preparation; × 430.

tion. These sources of radiation give a rapid decrease of the effective dose with increasing distance from the source of radiation. Furthermore, the marked side-scatter, which is inherent in such radiations of relatively low energy will give a gradation of effects in all the lateral directions. However, if one attends to the physical features of the experimental radiation employed, it is possible to show that the glial cells are adversely affected by the radiation. Globus,[8] in a study of the effects of gamma radiation from radon seed implants in the medulla oblongata of the dog, was able to show that very few, if any, compound granular cells or microglia appeared in the irradiated area undergoing necrosis about the radon seed implant, and that neuronophagia of the affected neurons was very slight. The degree of response of the astrocytes, as observed by Globus in his experiments, are at vari-

§ References 1 and 2.

ance somewhat with our observations, but when his findings are correlated with the marked variation in dose-distribution one obtains from a source of gamma radiation such as a radon seed implant, the differences in degree of response are readily

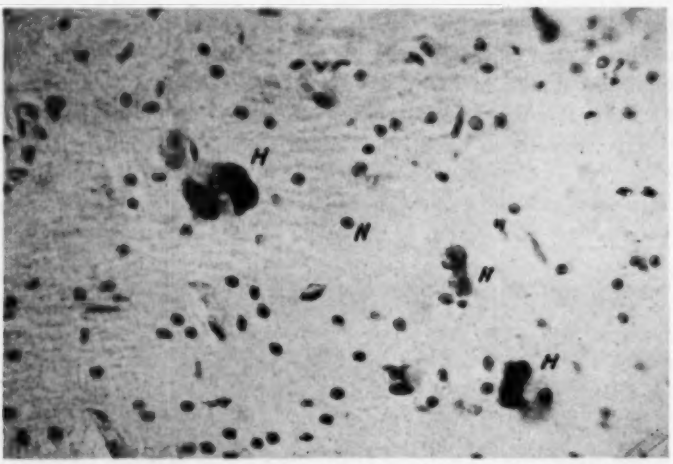

Fig. 3.—Variety of hypertrophied gial cells (*H*) and the stages of development of these cells. Weil-Van Gieson preparation.

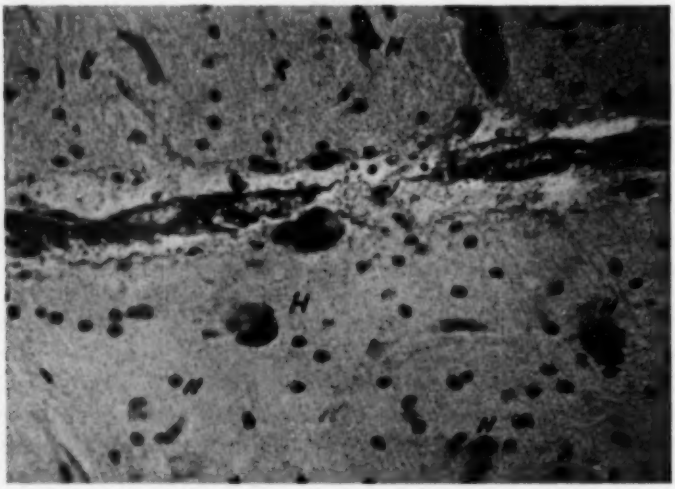

Fig. 4.—Variety of hypertrophied glial cells (*H*) and the stages of development of these cells. Weil-Van Gieson preparation; × 600.

accounted for. The bizarre degenerative hypertrophy of the glial cells which we have observed at long intervals of time after irradiation was not noted by Globus since his longest period of observation after implantation of the radon seed was 106 days. This unusual hypertrophy of the individual glial cells will now be considered.

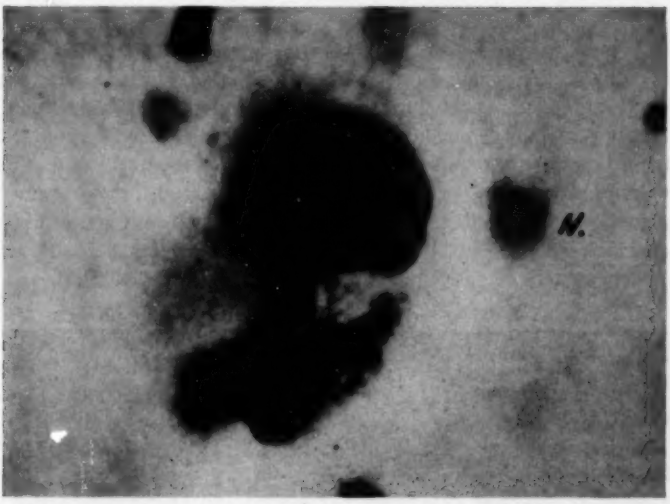

Fig. 5.—Variations in the morphology of the hypertrophied glial cells. Abnormal glial cell with a bilobed nucleus. *N* is a glial cell of normal size. Weil-Van Gieson preparation; × 1,200.

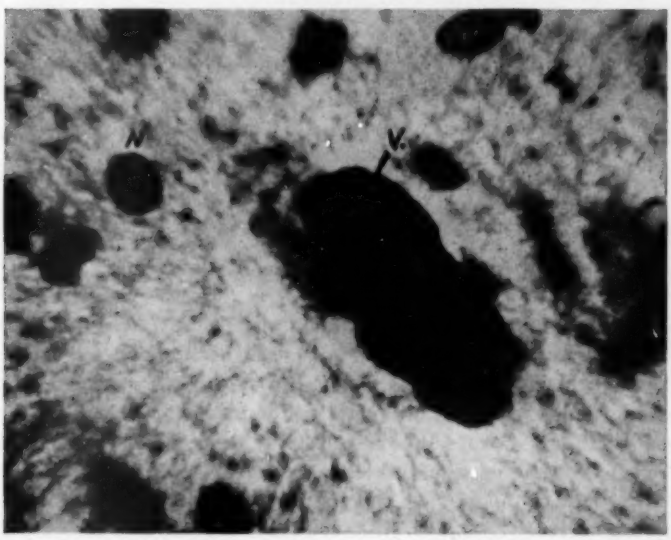

Fig. 6.—Abnormal glial cell with large nuclear vacuole and vacuolated cytoplasm. *V* indicates vacuoles. Weil-Van Gieson preparation; × 1,200.

4. *Glial Responses as Related to Duration of Time of Observation After Irradiation.*—It would appear that the early effects of radiation on the glial cells are inhibitory. However, with the passage of time, a recovery of glial function occurs which results in an intense gliosis. At times a very marked hypertrophy of the individual glial cells occurs at late intervals after irradiation which is quite striking in appearance. Figures 2, 3, and 4 show the hypertrophy of the individual glial cells in the molecular layer of the cerebellum of a patient who had received a course of x-ray therapy for a brain stem glioma some 15 months prior to death. These abnormal glial cells were observed only in the fields of irradiation, and they were unrelated to the brain stem glioma. Similar monstrous glial cells were observed in areas of the brain stem which were not involved by the tumor. In general these abnormal glial cells are approximately 10 to 20 times the size of the normal glial cell and possess

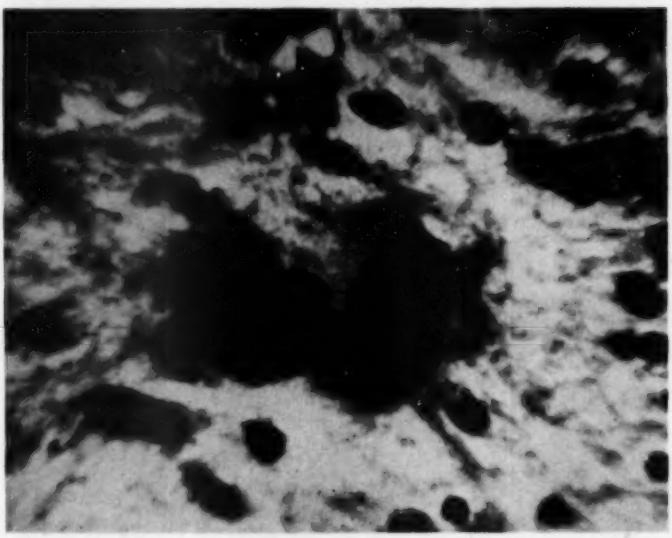

Fig. 7.—Abnormal gigantic binuclear glial cell. Weil-Van Gieson preparation; × 1,200.

rather grotesquely-formed, hyperchromatically-stained nuclei (Figs. 5, 6, and 7). Some of the nuclei are bilobed and have a band of nuclear material "restraining" the separation of the two nuclear lobes (Fig. 5). Such an abnormal cell may very well represent the end result of faulty glial cell division following irradiation. The phenomenon of faulty nuclear division (nuclear adhesiveness) with the resultant failure of cell division and subsequent cell hypertrophy has been noted in other tissues following irradiation. Some of the hypertrophied glial cells contain one or more discrete nuclear vacuoles (Fig. 6). These cells have very little or no cytoplasm and the cell outlines are usually quite irregular. Numerous cytoplasmic vacuoles are present at times. The various degree of development and comparative size of the glial cells can be readily observed in Figures 5, 6, and 7. These cells are definitely not radiation fibroblasts and have no relationship to the blood vessels.

Similar glial cell changes are being observed in other cases under study.

COMMENT

These alterations which have been observed in function and structure of the glial cells following irradiation lend themselves to certain very practical considerations. The hypertrophy of the glial cells observed after radiation would appear to be a degenerative phenomenon. Whether such altered cells will undergo neoplastic transformation remains to be determined. From a practical point of view, the appearance of such giant glial cells can be troublesome to the pathologist who has to examine biopsy material obtained from patients with central nervous system neoplasms who have had previous x-ray therapy. Such giant glial cell forms can appear in both the tumor and in the normal tissues. Their appearance in a slowly-growing tumor such as an astrocytoma could lead one to a diagnosis of a malignant lesion, since similar abnormal glial forms may be observed in nonirradiated malignant gliomas. At times, sufficiently large aggregates of these cells have been observed in irradiated normal tissues so that the examination of a small biopsy specimen of such tissues could be readily mistaken for a tumor recurrence, for which additional unnecessary x-ray therapy might well be given.

SUMMARY AND CONCLUSIONS

The normal adult glial cell of the primate brain can be adversely affected by irradiations.

The alterations in the function as well as the structure of the glial cells can be correlated with (*a*) the total dose of radiation, (*b*) the intensity of dose administration, (*c*) uniformity of dose-distribution within the tissue, and (*d*) the duration of time of observation after irradiation.

The clinical significance of these observations for the pathologist has been indicated.

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LOCAL EFFECT OF STAPHYLOCOCCAL TOXIN

Studies on Blood Vessels with Particular Reference to Phenomenon of Dermonecrosis

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MINNEAPOLIS

ONE OF the most characteristic properties of staphylococcal toxins derived from many pathogenic strains is the ability to induce necrosis of skin when injected intradermally. While this lesion was first described in 1924 by Julia Parker,[1] investigations into the mechanism of the phenomenon have been lacking, although some attention has been paid to other biological properties of the toxin,[2] such as its lethal * and hemolytic [5] effects and its ability to produce renal cortex necrosis [6] when injected intravenously into the experimental animal.

In man, local staphylococcal disease is commonly associated with extensive tissue destruction, and the same is true of staphylococcal diseases of lower animals. The possibility exists that the pathogenesis of experimental dermonecrosis may closely resemble that of naturally occurring necrotizing staphylococcal lesions. Indeed, the report by Stookey and co-workers [7] demonstrates the natural occurrence of an identical lesion in several human cases of staphylococcal skin infection. Furthermore, these authors were able to culture the offending organism and produce dermal necrosis by infiltrating the culture filtrate into the skin of rabbits. The studies reported herein demonstrate that the underlying mechanism of the dermonecrosis is a prolonged local ischemia and that the lesion is, in fact, an anemic infarction of the skin. Moreover, it will be shown that the toxin produces alterations in capillary permeability and prolonged vasospasm of small arteries and veins and that singly, or in the aggregate, these factors are responsible for the local ischemia and subsequent necrosis.

MATERIALS AND METHODS

Animals.—Adult rabbits weighing 2 to 4 kg. of both sexes and of mixed breeds were used, together with a number of mongrel dogs, weighing between 8 and 10 kg.

Toxin.—The staphylococcal toxin † used throughout these experiments was prepared from the Wood 46 strain and grown on the Leonard-Holme [8] medium by the method of Casman [9] with continuous agitation for 48 hours in an atmosphere of 80% oxygen and 20% carbon dioxide and then passed through a Berkefeld filter. The toxin was standardized in regard to two of its properties, the hemolytic and the dermal effect. The hemolytic potency of the toxin was titrated

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* References 3 and 4.

† Staphylococcus provided by Dr. H. D. Piersma, Lederle Laboratories, Pearl River, N. Y.

against the standard unit of antitoxin in the following way: A 0.5 cc. dilution of toxin was mixed with 1 unit of standard antitoxin contained in 0.5 cc. of 0.9% saline. A 1.0 cc. quantity of a 1% saline suspension of rabbit red cells was added and the mixture shaken and incubated in the water bath at 37 C. for one hour. The dilution showing 2+ hemolysis was taken as the end-point. The toxin used throughout our experiments showed 2+ hemolysis in the 1:8 dilution. The dermonecrotic standardization was carried out as follows: Three albino rabbits whose backs had been shaved at least 48 hours previously were injected intradermally with 0.1 cc. of varying dilutions of the toxin. The least dilution which caused a necrotic area 10 mm. in diameter after four days was taken as the minimizing necrotizing dilution. This was found to be 1:100.

Antitoxin.—The antitoxin used was of equine origin and contained 800 units per cubic centimeter.

Trypan Blue.—Twenty cubic centimeter amounts of a 1% saline solution of trypan blue was used throughout the experiments.

Heparinization.—The heparin solution used‡ contained 10 mg., or 1,000 units, per cubic centimeter. The substance was injected intravenously in a dosage of 3 mg. per kilogram and the clotting time, as determined by the modified Lee-White method, maintained at over 40 minutes. When the clotting defect had been established, it was maintained by means of depot heparin,§ doses of 50 mg. subcutaneously every five to six hours.

Transilluminator System: The system used was essentially a modification of the quartz rod technique of Knisely.[10] In place of the quartz rod, a 1 ft. (30 cm.) cylinder of Lucite, having a diameter of 1 in. (2.5 cm.) and bent in its distal portion to a right angle, was used to carry light from a 50 Watt microscope lamp. The tissues were supported on a Lucite platform and were kept moist and warm by a flow of Ringer's gelatin solution. Observations were made over prolonged periods using a binocular dissecting microscope.

Preparations and Staining of Sections.—Blocks of tissue were fixed in formol-saline solution and sections were stained by means of the hematoxylin and eosin method.

EXPERIMENTAL RESULTS

Anatomical Characteristics of Staphylococcal Dermonecrosis.—The intradermal injection of 0.2 cc. of 1:10 toxin dilution is followed by the immediate appearance of a 1 cm. raised wheal which rapidly disappears. By 10 minutes there is a 1.0 to 1.5 cm. area of hyperemia surrounding the site of injection. This enlarges progressively over a period of about two to four hours. At the end of this period the skin area begins to get increasingly pale, and by 12 hours the waxy yellowish pallor contrasts sharply with the surrounding normal skin. For the first 12 hours there is a marked tendency for the lesion to increase in size by lateral spread. By 24 hours the lesion, which may now vary from 3 to 8 cm. in diameter, consists of a central, pale, waxy area of skin surrounded by a well-demarcated raised serpiginous zone of hyperemia. By 48 hours, this zone of hyperemia is well marked and the central area becomes increasingly more desiccated. By 72 hours there is a well-demarcated, dark, brownish-red eschar which goes on to separation between 14 to 21 days after injection, leaving a punched-out, well-circumscribed ulcer (Fig. 4).

Microscopically, at one hour, virtually the only change seen is an engorgement of the capillaries and small venules. These vessels are crammed tightly with well-formed red blood cells. There is slight edema of the corium. At two hours, there is no demonstrable change in the histologic picture, but by three hours there are focal small hemorrhages and massive diapedesis of polymorphonuclear leucocytes through

‡ Parke, Davis & Company.

§ The Upjohn Company, Kalamazoo, Mich.

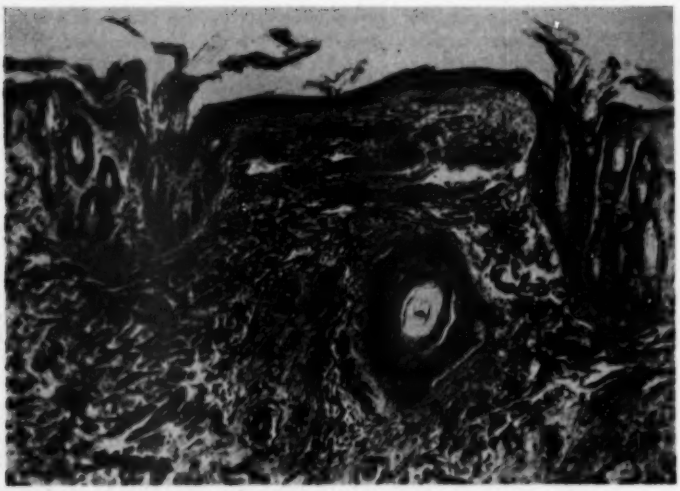

Fig. 1.—Staphylococcal dermonecrosis 24 hours after intradermal injection of 0.2 cc. of 1 : 10 toxin dilution. The epithelial layer shows early necrosis while inflammatory cell infiltration is conspicuously absent from the underlying corium.

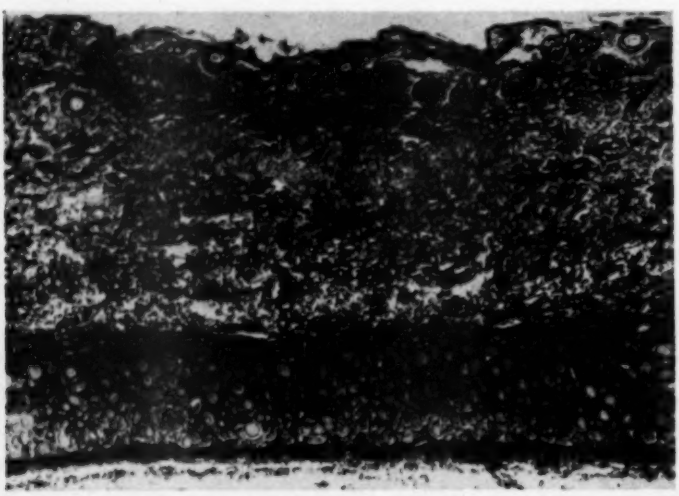

Fig. 2.—Low power view of the same section showing a band of polymorphonuclear leucocytes advancing through the platysma muscle into the necrotic area.

the walls of the small blood vessels in the skin, at the boundary-line between the site of injection and the surrounding normal skin. The vessels of the central zone retain their engorged appearance and are filled with red cells, which at this time appear less well-defined and more closely packed. The overlying epithelium appears entirely normal. By 24 hours, the epithelial cells show obvious signs of necrosis, their nuclei being pyknotic and their cytoplasm reduced to an eosinophilic coagulum (Figs. 1 and 2). The fibrous tissue of the underlying corium is swollen and intensely eosinophilic. There are focal small interstitial hemorrhages, and the venules and capillaries retain their state of intense engorgement though at this period the endothelial lining cells are swollen and basophilic. In some cases, the swelling of the endothelial lining cells in the capillaries is of such proportions as to occlude the lumen completely. The intimal and medial layers of the arterioles and small arteries in some areas show swelling, hyalin necrosis of the muscular media with occasional infiltration of polymorphonuclear leucocytes into the media and adventitia. One of the most striking features of this extensive area of early dermal necrosis is the paucity of inflammatory-cell infiltration. Indeed, inflammatory cells may be completely absent in the area of necrosis (Fig. 1). This contrasts sharply with the boundary zone where an intense acute inflammatory reaction with extensive polymorphonuclear infiltration is evident. At this stage, the picture is that of a central area of infarction surrounded by a well-defined band of infiltrating polymorphonuclear leucocytes. By 72 hours, the epithelial layer is transformed into a basophilic mass of pyknotic and karyorrhectic nuclei surmounting a completely hyalinized eosinophilic corium, in which the remnants of coagulated blood vessels engorged with amorphous masses of destroyed red cells are evident. There are focal scattered hemorrhages and, at this stage, extensive polymorphonuclear invasion both from the deeper fascial and muscular layers of the abdominal wall and from the peripheral boundary zone, both of which are edematous and intensely inflamed. Ultimately, the necrotic tissue sloughs away from the underlying inflamed tissue, the plane of separation occurring in the zone of polymorphonuclear infiltration. It should be noted that hyalin thrombosis of small vessels is not a feature of this lesion, though it is occasionally seen in the depths of the lesion during the 24 to 48 hour period.

LOCAL EFFECTS OF STAPHYLOCOCCAL TOXIN ON MESENTERIC BLOOD VESSELS

In a previous paper,[11] it was shown that staphylococcal toxin given intravenously in the experimental animal produces prolonged and selective spastic occlusion of the renal vascular system, which, in some animals, was of such duration as to produce symmetrical cortical necrosis of the kidneys. It seemed possible, therefore, that similar vascular effects were responsible for the dermonecrosis, especially since microscopic examination indicated that the lesion was essentially an infarction of skin. To explore this thesis further, the direct effect of staphylococcal toxin infiltrated around small mesenteric blood vessels was studied in transilluminated preparations in both dogs and rabbits.

With pentobarbital (Nembutal) anesthesia, a midline laparatomy incision was made, a loop of ileum withdrawn, and an area selected where fat accumulation about the mesenteric blood vessels was minimal. This area of mesentery was then arranged on the transilluminator system in such a way that a group of relatively straight

arteries and veins was in the center of the field. Ringer gelatin drip was then started, to prevent desiccation of the tissue, and a binocular dissecting microscope brought to bear on the field. Control studies, during which a single group of blood vessels was observed for several hours, showed no alteration in vessel caliber. During these long periods of control observation, the efficiency of the preparation was shown by the fact that the artery continued to pulsate vigorously and the red cells in the capillary channels could be seen in rapid flow. Prior to each experiment, the vessels were observed for a control period of 20 minutes, at the end of which 0.05 cc. of toxin dilution was injected through a fine needle into the areolar tissue between artery and vein, care being taken that neither vessel was injured, and observation was then continued.

In this study, various toxin concentrations ranging from undiluted toxin to 1 : 50 saline dilution were used. In each experiment, a pronounced segmental spasm of both artery and vein occurred within a few minutes after toxin infiltration. The

TABLE 1.—*The Vasospastic Effects of Staphylococcal Toxin Infiltrated Around the Mesenteric Blood Vessels*

Animal	No. of Observations	Toxin Concentration	Onset of Spasm	Duration of Spasm
Dog	10	Undiluted	2-4 min.	4 hr. or longer
	10	1:10	2-4 min.	2-4 hr.
	5	1:40	4-8 min.	1-1½ hr.
Rabbit	10	Undiluted	30-120 sec.	4 hr. or longer
	10	1:10	1-2 min.	4 hr. or longer
	5	1:40	4-6 min.	40-90 min.
Control dog	5	Undiluted toxin inactivated by heating to 100 C. for 1 hr.	No effect on vessels	0
	5	Undiluted toxin inactivated with specific antitoxin	No effect on vessels	0

spasm was of such degree, in many instances, as to make it appear unlikely that blood flow could continue through the spastic area. Within 20 to 30 minutes, the area of spasm which originally occupied a distance of about 0.5 cm. had spread both proximally and distally for 1 to 2 cm., and pulsation in the particular artery under study had ceased completely, though arteries in adjacent ileal arcades continued to pulsate vigorously. A similar effect was noted on the adjacent vein. Indeed, generally, the veins were more severely affected.

Observation was continued for several hours and spasm was found to persist unabated for from two to four hours. The preparations were abandoned after four hours. The duration of spasm was directly related to the toxin concentration used. At the end of this period, the vessel gradually returned to normal in many animals, and about 30 minutes after the spasm had begun to relax vessel caliber was restored and, in the case of the artery, pulsation was once again apparent. The findings are summarized in Table 1.

The effect of toxin on capillary vessels was then tested by applying tiny sponges saturated in toxin directly to the vessel for about 30 seconds. The sponge was then removed and observations continued. For about five minutes the normal active circulation continued; then it was noticed that the erythrocytes, which previously

had been well-dispersed in fluid medium, now appeared more concentrated. This hemoconcentration progressed over the subsequent two to three minutes, and at the same time the circulation through the capillary became increasingly more sluggish until it ceased, the vessel being crammed tightly with red cells. No alterations in vessel caliber was noted. It would seem that the action of staphylococcal toxin on the capillary vessels is to alter membrane permeability to such an extent that local plasma loss results in packing of red cells, with consequent obstruction to capillary blood flow.

TABLE 2.—*The Effect of Intradermal Infiltration of Staphylococcal Toxin on Cutaneous Blood Flow as Revealed by the Intravenous Trypan Blue Test*

Number of Rabbits	Substance Injected Intradermally	Time Interval Between Intradermal Injection and Trypan Blue	Results of Dye Injection	
			Number of Rabbits Showing Diffusion of Trypan Blue into Infiltrated Area	Number of Rabbits Showing Complete Exclusion of Dye from Infiltrated Area
4	0.2 cc. 1:10 staphylotoxin	10 min.	4 Intense staining of infiltrated area	0
5	0.2 cc. 1:10 staphylotoxin	20 min.	2	3
4	2.0 cc. 1:10 staphylotoxin	1 hr.	0	4
4	2.0 cc. 1:10 staphylotoxin	2 hr.	0	4
4	2.0 cc. 1:10 staphylotoxin	4 hr.	0	4
4	2.0 cc. 1:10 staphylotoxin	24 hr.	0	4
4	0.2 cc. xylol	2 hr.	4 Intense staining of infiltrated area	0
4	0.2 cc. meningococcal toxin	2 hr.	4 Intense staining of infiltrated area	0
2	0.2 cc. saline	10 min.	Uniform blue staining of skin	0
2	0.2 cc. saline	1 hr.	Uniform blue staining of skin	0
2	0.2 cc. saline	2 hr.	Uniform blue staining of skin	0
2	0.2 cc. saline	4 hr.	Uniform blue staining of skin	0
2	0.2 cc. saline	24 hr.	Uniform blue staining of skin	0
4	0.2 cc. heated staphylotoxin	2 hr.	Uniform blue staining of skin	0

From these observations it would seem that staphylococcal toxin infiltrated into living tissue produces a local diminution in blood flow by two mechanisms: first, by causing prolonged and severe spasm in vessels possessing a muscular coat and, second, by altering capillary permeability and so producing local circulatory stasis.

ROLE OF PROLONGED LOCAL ISCHEMIA IN THE CAUSATION OF THE DERMONECROSIS

From the histological observations and the studies of the direct effects of toxin on mesenteric blood vessels it appeared likely that the dermal necrosis was the result of prolonged local ischemia. The following experiment was carried out to determine the presence or absence of blood flow to the area of skin previously infiltrated with toxin: Twenty-five rabbits which had been shaved at least 24 hours previously each received an intradermal injection of 0.2 cc. of a 1:10 saline dilution of toxin. The animals were then divided into six groups and the state of blood flow in the area of infiltration tested by the intravenous administration of 20 cc. 1% trypan blue at

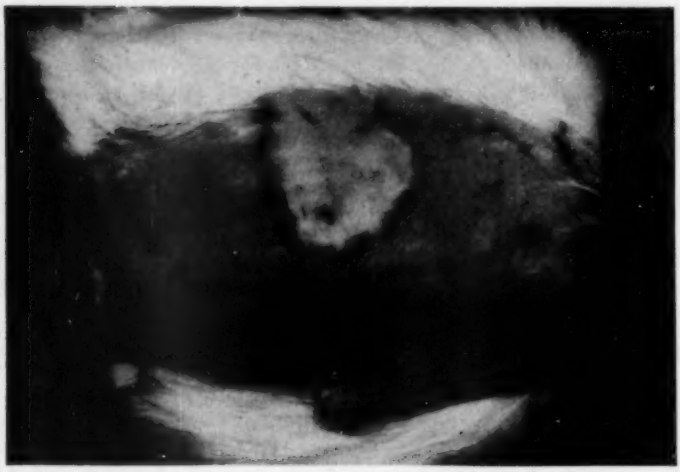

Fig. 3.—Staphylococcal dermal lesion 24 hours after the intradermal injection of 0.2 cc. of 1 : 10 toxin dilution. This animal received 20 cc. of 1% trypan blue 15 minutes prior to being photographed. The area of toxin spread maintained its white color contrasting with the remainder of the skin which took on a uniform blue color.

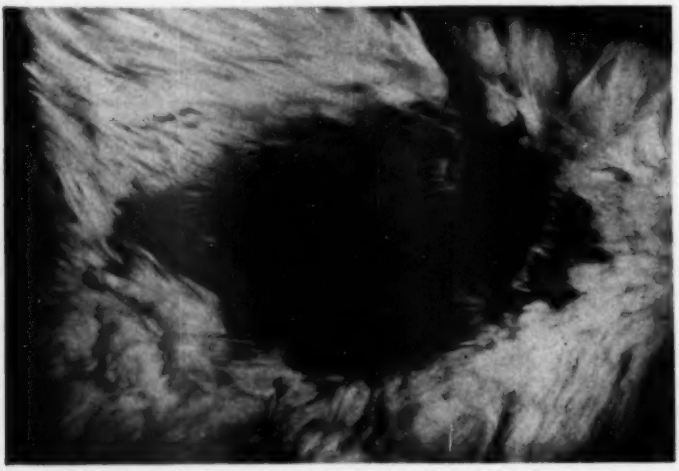

Fig. 4.—Staphylococcal dermonecrosis 10 days after the intradermal injection of 0.2 cc. of 1 : 10 toxin dilution. The skin area previously excluded from blood flow is represented by a thickened eschar.

specified intervals. Since trypan blue is initially protein-bound and circulates in the plasma for at least 20 minutes, the presence or absence of blue coloration in the skin is an index of blood flow. The results, which are summarized in Table 2, were read 15 minutes after injection of the dye. It will be seen that all animals tested during the first 10 minutes after the adminstration of toxin showed an intense blue color at the site of infiltration. At 20 minutes three of the five animals tested showed complete exclusion of blue dye from a 1 to 2 cm. area of skin surrounding the injection site. The remainder showed an excessive deposition of blue dye in and around the site of injection. On the other hand, at one hour and at all subsequent periods the results uniformly showed that the area of infiltration and spread of the toxin remained absolutely white and unchanged (Fig. 3), while the surrounding skin stained blue and a particularly bright blue in the serpiginous inflammatory zone surrounding the area of toxin infiltration. At 72 hours, all the animals infiltrated with toxin showed obvious areas of dermonecrosis varying from 3 to 8 cm. in diameter.

These results consistently demonstrated that, apart from the initial 10 to 20 minute period when a state of local increased capillary permeability existed, the area of toxin infiltration and spread was completely excluded from blood flow for at least 24 hours, by which time histological evidence of infarction was obvious. It should be noted that no such area of dye exclusion could be observed in 10 control animals which were infiltrated with 0.2 cc. of isotonic sodium chloride solution in place of toxin. In these animals uniform blue staining of the skin was consistently found. This was also true of four animals in which toxin inactivated by heating to 100 C. for one hour was infiltrated. Further control studies were performed to show the contrast in blood flow to areas of acute dermal inflammation produced with xylol and Meningococcus toxin respectively. In both areas, as shown in Table 2, there was rapid and intense blue coloration of the inflamed area. This conforms with the findings of Menkin,[14] who, using the trypan blue technique, demonstrated increased vascularity and capillary permeability in areas of acute inflammation. Staphylococcal toxin, on the other hand, produces a diametrically opposite effect.

EFFECTS OF HEPARINIZATION ON STAPHYLOCOCCAL DERMONECROSIS

While there is no gross or histological similarity between the dermal Shwartzmann reaction ‖ and staphylococcal dermonecrosis except in the end-result, and while thrombosis is a constant feature in the early lesion of the former and completely absent in the early lesion of the latter, yet it seemed desirable to test the effect of heparinization on the development of staphylococcal dermonecrosis if only to provide further evidence that thrombosis is not of etiological importance in this phenomenon.

Four rabbits having control clotting times varying between 40 and 120 seconds received 3 mg. per kilogram of body weight of heparin sodium ¶ intravenously. Clotting times were determined by the modified Lee-White method at hourly intervals and the dose repeated when the clotting time fell below 40 minutes. Depot

‖ References 12 and 15.

¶ Parke, Davis & Company.

heparin given subcutaneously in 50 mg. doses was used to maintain the clotting defect once the desired level had been attained. In general, this dose of depot heparin maintained the level satisfactorily for six to eight hour periods.

When the animals were fully heparinized, each received an intradermal injection of 0.2 cc. of 1 : 10 staphylococcal toxin. The area of infiltration was then observed. In all four animals, dermonecrosis developed in an identical manner to that of the control animals. Two rabbits were allowed to survive until large eschars formed, while the other two were killed at 48 hours and sections of skin taken. Histological studies showed the same picture of a well-demarcated cutaneous infarction noted in the control animals.

THE INFLUENCE OF SUBSEQUENT LOCAL ADMINISTRATION OF SPECIFIC ANTITOXIN

When staphylococcal toxin is infiltrated intradermally in the rabbit, the reaction proceeds relentlessly to local gangrene. Hence, it seemed of importance to study the modification of this reaction by post hoc infiltration of specific antitoxin and by so doing to determine the time interval at which the reaction could be either completely aborted or controlled. Accordingly, the following experiment was carried out: Ten rabbits were shaved extensively on both abdomen and back. Intradermal injections of 0.2 cc. of 1 : 10 toxin were then made on two widely separated areas on the back and one area on the abdomen—in all, three injections in each animal. At varying intervals thereafter, 5 cc. of 1 : 100 saline dilution of antitoxin was infiltrated into and around each injection site on the back. Only one dose of antitoxin was given at each injection site. The abdominal area served as a control and was infiltrated with 5 cc. of isotonic sodium chloride solution. It should be noted that the amount of antitoxin used far exceeded that needed to neutralize the toxin as judged by the hemolytic titration test.

Twenty-four hours after toxin injection, trypan blue was injected intravenously to compare cutaneous blood flow in the areas of toxin-antitoxin infiltration and the control area of toxin-saline infiltration.

The results are summarized in Table 3, whence it will be seen that antitoxin infiltrated within 10 minutes of toxin injection completely prevented the development of cutaneous ischemia and of dermonecrosis. Antitoxin infiltration at 20 minutes gave an identical result to that at 10 minutes in one test and a tiny area of persistent ischemia and subsequent dermonecrosis at the site of injection in the remaining three tests. At the one hour interval, the results were very similar to those at the 20 minute interval, the area of ischemia and necrosis generally being slightly larger, though contrasting sharply with the huge area of necrosis at the control site. Antitoxin injection at two hours showed a corresponding increase in both the area of ischemia and dermonecrosis to a maximum of 2 cm. though here again the control areas were two to three times as large, showing that the antitoxin had inhibited to a limited extent the natural tendency of these lesions to lateral spreading. By contrast, antitoxin infiltration at the four hour interval had but slight inhibitory effect, and at 24 hours no effect, on the area of subsequent dermonecrosis was obtained.

One of the most striking features of this study was the precise correlation between the area of dye exclusion at 24 hours and the area of sloughing grossly evident at 72 hours.

TABLE 3.—*Effect of Subsequent Antitoxin Infiltration on the Local Blood Flow and the Area of Dermonecrosis*

Interval Between Toxin Injection and Antitoxin Injection	Number of Skin Tests	Effect of Intravenous Trypan Blue 24 Hours After Toxin Injection		Dermonecrosis at 72 Hours		Comment
		Antitoxin Infiltration	Saline Control	At Site of Antitoxin Infiltration	Saline Control	
10 min.	4	Diffuse uniform blue at site of injection and surrounding skin in all animals	4-8 cm. area of yellow-white dye exclusion; neighboring skin uniformly blue	None in all animals	4-8 cm. area of dermonecrosis in all animals	Complete protection from dermonecrosis and prevention of local ischemic effect
20 min.	4	Diffuse blue in 1 animal. 0.5 cm. area of dye exclusion in 3 animals	4-8 cm. area of yellow-white dye exclusion; neighboring skin uniformly blue	None in 1 animal; 0.5 cm. area of necrosis in 3 animals	4-8 cm. area of dermonecrosis in all animals	Tiny area of ischemia with subsequent necrosis at injection site; considerable inhibition of dermonecrotic effect
1 hr.	4	0.5-1 cm. area of dye exclusion in all animals	4-8 cm. area of yellow-white dye exclusion; neighboring skin uniformly blue	0.5-1.0 cm. area of necrosis in all animals	4-8 cm. area of dermonecrosis in all animals	Slightly increased area of ischemia with correspondingly increased area of necrosis; considerable inhibition of dermonecrotic effect
2 hr.	4	1-2 cm. area of dye exclusion in all animals	4-8 cm. area of yellow-white dye exclusion; neighboring skin uniformly blue	1-2 cm. area of necrosis	4-8 cm. area of dermonecrosis in all animals	Increasing areas of ischemia and dermonecrosis; moderate inhibition of dermonecrotic effect
4 hr.	4	3-4 cm. area of dye exclusion in all animals	4-8 cm. area of yellow-white dye exclusion; neighboring skin uniformly blue	3-4 cm. area of necrosis corresponding precisely to excluded area	4-8 cm. area of dermonecrosis in all animals	Areas infiltrated with antitoxin show smaller lesions and less tendency to lateral spread; slight inhibition of dermonecrotic effect
24 hr.	4	4-8 cm. area of dye exclusion in all animals	4-8 cm. area of yellow-white dye exclusion; neighboring skin uniformly blue	4-8 cm. area of necrosis corresponding precisely to excluded area	4-8 cm. area of dermonecrosis in all animals	No inhibitory effect

COMMENT

The discovery by Julia Parker [1] in 1924 of the dermonecrotic effects of staphylococcal culture filtrates has important implications in the study of the naturally occurring disease, for it exemplifies in an easily accessible form the local necrotizing action which so frequently characterizes staphylococcal infection.

The pathogenesis of the dermonecrosis has been assumed to be due to a hitherto undefined necrotizing action of the toxin on living cells.[13] The possibility exists that such activity resides in the demonstrated proteolytic effects of the toxin. Yet a potent argument against this thesis is the finding that extensive dermonecrosis is produced by high dilutions of toxin while trypsin solutions, possessing greater proteolytic activity, fail to produce comparable skin lesions.# The necrotizing effects of the toxin, which are fundamentally similar in the diverse organs into which it has been injected, are better explained by a consideration of its local ischemic effects.

A number of facts make it appear quite likely that staphylococcal toxin produces pronounced vascular effects when administered intravenously. First, a study of the lethal mechanism by Kellaway, Burnet, and Williams [4] indicates that the toxin produces an obstruction to the pulmonary circulation and acute right heart failure in rabbits. Second, numerous experimental studies of sublethal doses show an ischemic effect on the kidneys producing symmetrical cortical necrosis.[6] Further studies demonstrate that the renal effects are due to prolonged and selective renal vasospasm.[11] These studies suggested that staphylococcal dermonecrosis might be the result of prolonged cutaneous ischemia. The investigations reported in the present work bear out this hypothesis.

A consideration of the gross and histological changes has considerable bearing on the problem. Grossly, apart from an initial transient hyperemia, the affected skin area becomes pale and dry, and by 24 hours a raised acute inflammatory zone of demarcation outlines its borders. Ultimately, the whole affected area sloughs. In effect, the pathological process greatly resembles a dry gangrene. Histologically, too, the process in no way resembles the usual acute inflammatory reaction. Indeed, the most striking feature of the disease is the continued absence of inflammatory cells or vascular reaction in the skin area destined to undergo necrosis. By contrast, the boundary zone shows a marked inflammatory reaction. That the mechanism underlying the dermonecrosis is indeed an ischemic one is further borne out by the intravenous trypan blue studies, which show that, after a brief increase in local capillary permeability, there supervenes a prolonged period of dermal ischemia which persists until frank dermal necrosis occurs. This contrasts with the usual type of inflammatory reaction, where, as shown by Menkin,[14] the increased local circulation and altered capillary permeability is manifested by the excessive diffusion of intravenously injected trypan blue into the inflamed area.

Direct observations of the mesenteric blood vessels show that toxin produces severe and prolonged segmental spasm of arteries and veins and alterations in capillary permeability which result in local hemoconcentration and stasis of blood flow. Furthermore, histological studies demonstrate that thrombosis is not a feature of the dermonecrosis during the first 24 hours. Engorgement of capillary vessels with red cells is a common histological feature of the developing dermonecrosis and is also readily evident on direct observation of living capillaries to which toxin has

Thal, A. P., and Egner, W.: Unpublished data.

been applied. Certainly, it would seem that the packed masses of red cells distending the capillaries may act as a local barrier to circulation, yet this does not constitute thrombosis in the accepted sense of the word. Moreover, heparinization, which prevents the fibrin-platelet thrombosis of the Shwartzmann phenomenon,[18] is wholly without effect on staphylococcal dermonecrosis. From the facts available, it would seem that the ischemia is the combined result of prolonged spasm of the larger vessels and stasis of flow in capillary vessels due to local plasma loss.

While the experiments reported above, utilizing a sterile culture filtrate, are not comparable to natural infection, yet they emphasize an ischemic effect which may play an important part in local staphylococcal disease. In this regard, the disease of dairy cattle known as gangrenous staphylococcal mastitis is of some interest. Occurring chiefly at the beginning of lactation, this condition affects one or more quarters of the udder and within 48 hours may proceed to total gangrene of the affected area. A most striking clinical finding is the well-demarcated area of coldness which precedes the gangrene by about 24 hours and accurately outlines the quarter destined to become gangrenous.* It is difficult to escape the conviction that an ischemic mechanism is operating in this disease and possibly in other staphylococcal infections characterized by massive tissue destruction.

SUMMARY

The gross and microscopic features of dermonecrosis induced in rabbits with staphylococcal toxin were studied and it was found that the pathological changes indicated an infarction of the skin. That the skin area destined to become necrotic was indeed excluded from the systemic circulation was shown by intravenous trypan blue studies. Direct observation of the mesenteric blood vessels in the living dog and rabbit showed that staphylococcal toxin produced prolonged segmental spasm of small arteries and veins when the toxin was infiltrated around them. Capillary permeability was altered in such a way that stagnation of blood flow resulted. It was postulated that these two mechanisms acting together were responsible for the local dermal ischemia and subsequent necrosis. Further studies showed that the dermonecrosis was unaffected by heparinization and that infiltration of specific antitoxin up to two hours after toxin injection exercised an inhibitory effect on the dermonecrosis.

The implications of these findings for naturally occurring necrotizing staphylococcal disease are briefly discussed.

The photographs were made by Mr. Lloyd Wolf.

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NEPHROTOXIC GLOBULIN NEPHRITIS

V. Effects of Adrenal Steroid Administration or Adrenalectomy

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THE COURSE of experimental nephritis, produced in the rat by administration of rabbit anti-rat-kidney gamma globulin (NTG) or by crude rabbit anti-rat-kidney serum, is subject to the influence of endocrine functions. The administration of desoxycorticosterone acetate (DCA) with liberal salt consumption has been reported to increase the renal size and increase the blood pressure beyond control values in nephrotoxic serum nephritis.[1] It has been reported that cortisone failed to prevent, or produced a slight increase in, the severity of nephrotoxic serum nephritis,* or, in other hands, produced a more pronounced increase in the severity of the nephritis.[6] Corticotropin (ACTH) has been reported to increase the severity of the nephritis in varying degrees.† Adrenalectomy has been reported to diminish the proteinuria after nephrotoxic serum administration.[5] A relationship between adrenal size and severity of the nephritis has been indicated.‡

However, the effect of certain biological variables (age, sex, route of NTG administration, dose and potency of NTG) upon the course of NTG nephritis is pronounced.[8] Since the conclusions of previous work were not all consistent, and since most of the reports concerning endocrine influences upon NTG nephritis failed to control all of the factors mentioned above, this study was undertaken to define more precisely the effect of adrenal steroid administration and adrenalectomy upon the manifestations of NTG nephritis in the rat.

METHODS AND MATERIALS

Nephrotoxic Globulin (NTG).—Purified nephrotoxic gamma globulin was prepared from rabbit anti-rat-kidney serum in the manner previously described.[9] A single lot (NTG 8/9/51) was used for all the steroid administration experiments. Each animal was given a single intra-

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena; Contribution No. 1865.

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* References 2 and 3.

† References 2 and 4.

‡ References 3 and 7.

venous dose, 6.9 mg. of total protein in a volume of 0.3 ml., measured in a tuberculin syringe. A different single lot (NTG 1/3/52) was used for the adrenalectomy experiment, and each animal was given a single intravenous dose, 14.7 mg. of total protein in a volume of 0.3 ml. The minimal precipitable antibody content, measured with the saline-soluble kidney material as the precipitating antigen, was 13.5% for NTG 8/9/51 and 16.0% for NTG 1/3/52. The latter values do not indicate the total anti-kidney potency, since it cannot be assumed that all the antibodies are precipitable.

Experimental Procedure.—In these experiments, 104 rats of the Slonaker-Addis strain were used; all were adult animals, selected to weigh 150 gm. with little variation at the start of an experiment. Groups of 6 to 18 animals were used in each experiment. The animals that died as a result of the initial shock after NTG administration were discarded from the study, with the assumption that these immediate deaths were the consequence of antibodies that were not specific for the kidney.[10] Approximately 10% of the injected animals died within four hours after NTG administration in this series of experiments.

In all the steroid administration experiments identical procedure was followed. At the start of each experiment a blood pressure determination was made by the tail-microphone method of Friedman and Freed,[11] and a four-hour urine collection was made to determine the basal rate of protein excretion. Before and during the experiments, the animals were fed the colony stock diet,[12] which contained 17% protein and 0.48% sodium chloride, and tap water was given ad libitum. During the periods of urine collection the animals received no solid food, but only 10% dextrose solution with 0.4% sodium chloride and 0.5% vitamin B complex (Betaplexin).

The subcutaneous injections of adrenal steroids § were given daily, six days a week, until termination of the experiments after four weeks. The groins, flanks, and shoulders were used in rotation for the site of the subcutaneous injections. Intraperitoneal injections were given on the same schedule. The daily dose of each steroid was measured in a tuberculin syringe. Cortisone acetate was given suspended in a prepared aqueous menstruum,[13] and the daily dose was 2.5 mg. in a volume of 0.1 ml. The menstruum contained 1.5% benzyl alcohol, 0.9% sodium chloride, 0.4% polysorbate 80 (polyoxyethylene [20] sorbitan mono oleate), and 0.5% sodium carboxymethylcellulose. Hydrocortisone acetate[14] and desoxycorticosterone acetate[15] were suspended in an aqueous menstruum, and the daily dose was 2.0 mg. in a volume of 0.2 ml. The menstruum contained 0.85% sodium chloride, and it was found that a reasonably stable suspension could be produced by incorporation of 0.6% polysorbate 80 and a trace of caprylic alcohol. Preliminary experiments showed that the suspending agents had no effect in the dosages used, at least with respect to the principal manifestations of nephritis. For this reason, and because of the minor differences in amount which, if rigidly constructed, would have necessitated the use of several control groups, no injections of the vehicle were used in the single control group.

Blood pressure determinations, followed by four-hour urine collections for total protein determinations, were made at weekly intervals during the four-week period of each experiment. At the end of the fourth week the animals were killed by exsanguination from the severed abdominal aorta and vena cava during light ether anesthesia.

The following data were obtained at autopsy: wet weight of kidneys, heart, adrenal glands, gonads, and liver. The organ weights were compared with the value predicted for a normal rat of the same size by Addis and Gray. The presence of ascites was noted, and, if present, the quantity of fluid was measured. The total drawn blood volume was measured, and the serum was examined for visible lipemia, which, when present, was graded from 1+ to 4+. The tissues were fixed, stained, and sectioned for microscopic examination.

Protein content of the individual urine specimens was determined by the method of Shevky and Stafford.[16] Pools were made from aliquot portions of the urine and serum specimens in each group. Serum and urine urea determinations were performed by the method of Kibrick and Skupp.[17] Serum and urine total creatinine chromogen determinations were performed by the method of Bonsnes and Taussky.[18] Endogenous urea and creatinine chromogen clearances

§ Cortisone acetate and hydrocortisone acetate were supplied by Merck & Company, Inc., Rahway, N. J. Desoxycorticosterone acetate was supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

were calculated in the usual manner from the serum and urine values determined. Serum and urine total protein determinations were performed by the biuret method of Kingsley.[19] Serum total cholesterol determinations were performed by a slight modification of the method of Bloor, Pelkan, and Allen.[20] Serum total lipid determinations were performed by the method of Bloor.[21]

It was necessary to modify procedure for the group subjected to adrenalectomy, since the adrenalectomized animals could not be maintained in good general condition for four weeks after NTG administration. This group was given the intravenous dose of NTG, and 48 hours later both adrenal glands were removed through small flank incisions with light ether anesthesia. The upper pole of the kidney was exposed by retraction with a nasal speculum, and the entire adrenal gland was avulsed with an iris fixation forceps. Very little hemorrhage resulted, and the avulsed gland was inspected in order to be certain that it had been removed in toto. The wound was then closed with a single, continuous, silk suture. The operation was completed on both sides in approximately seven minutes, and immediately after the operation the animals seemed to be well. For control of this experiment, animals were subjected to sham adrenalectomy 48 hours after NTG administration. In the sham procedure the adrenal gland was handled gently and returned to its original position. After operation, the adrenalectomy and sham-adrenalectomy groups were given the usual stock diet, but they were given 0.85% sodium chloride solution to drink, instead of tap water. The urine was collected, and the animals were killed on the ninth day after NTG administration, one week after operation. At the autopsy, performed in the manner described for the other groups, the site of the adrenal gland was again examined for evidence of incomplete removal or regeneration of a gland fragment. In no animal was any remaining adrenal tissue observed, nor was any accessory adrenal tissue found, although accessory adrenal tissue is said to occur frequently in the rat.

RESULTS AND COMMENT

General Observations.—The general behavior and appearance of rats after the intravenous administration of NTG have been described in detail.‖ The same observations and comments in general pertain to these experiments, and the changes in behavior were grossly related to the severity of the nephritis, as graded by previously established criteria.[7] As mentioned, rats which were subjected to adrenalectomy after NTG administration were difficult to maintain in good condition, a result which conforms to the experience of Knowlton and co-workers.[8] Although few died during the first week after adrenalectomy in a pilot experiment, the death rate rose rapidly after one week. The animals seemed well until they were found dead in the cage, and no obvious cause of death was noted at autopsy. Since most of the animals seemed well during the first week, the experiments with adrenalectomy were terminated nine days after NTG administration, one week after adrenalectomy.

The unwieldy mass of data accumulated has been simplified by omission of data and discussion that did not contribute new information, but merely confirmed material previously reported. Microscopic examination of tissue sections revealed minor differences between the groups, if animals in the same grade of severity were compared. These differences were in the direction that might be expected; for example, with cortisone and hydrocortisone administration less collagen deposition might be expected. However, the differences were so slight as to be of doubtful significance.¶

Effects of Subcutaneous Adrenal Steroid Administration in the Male.—Desoxycorticosterone Acetate (DCA): Administration of DCA, in dosage comparable to

‖ References 7 and 9.

¶ Dr. Harry Goldblatt, The Mount Sinai Hospital, Cleveland, reviewed some of the tissue sections and independently confirmed the authors' conclusions.

that which others have used to produce hypertension in the rat, with [1] or without # concomitant administration of NTG, had no effect upon the magnitude of proteinuria one week after NTG administration. There was a slight but significant * increase in the proteinuria at four weeks (Chart 1). When the course of NTG nephritis is examined,[9] it is found that one week after NTG administration tissue sections of the kidney show maximal evidence of degeneration and inflammation, with relatively little evidence of collagenization or repair. On the other hand, four weeks after NTG administration, degeneration and inflammation have subsided, while glomerular collagenization and repair of tubular degeneration have made considerable progress. Thus, it might be inferred, that the influence of DCA given subcutaneously, though slight, is most prominent during the period of repair. The point grade of nephritis severity [7] was somewhat greater than the control grade after DCA administration as a consequence of an increase in the relative kidney and heart size (Table 1). No clear reason for the increase in relative kidney size could be found in the microscopic examination of tissue sections. Cardiac enlargement in

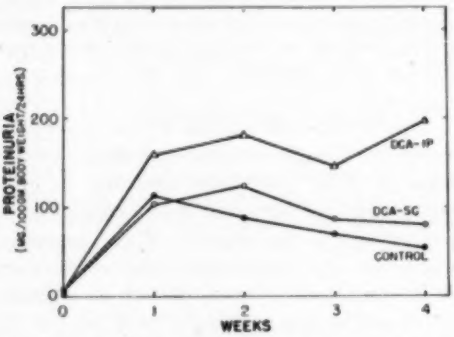

Chart 1.—Effect of subcutaneous and intraperitoneal DCA on proteinuria of NTG nephritis in the adult male rat.

NTG nephritis is related roughly to the increase in blood pressure. The DCA group had a slight increase in the blood pressure elevation after four weeks, when compared to the control group. The differences in relative liver and gonad weight were not considered significant; there was a slight but significant decrease in the relative adrenal gland weight after DCA administration. An over-all effect upon body growth was reflected in the final body weight, which was less in the DCA group than in the control group. No effect of DCA administration was noted in the serum total cholesterol or serum total lipid concentrations (Table 2). The serum urea and creatinine chromogen concentrations were significantly lower in the DCA group, but

References 22 and 23.

* Statistical analysis was not employed when the group differences in any respect were of obvious significance or insignificance. Where any doubt remained after inspection of the values, the significance of the difference was examined by the method of Fisher.[24] When such a difference is described as significant, the value for P was less than 0.01. A value for P of 0.01 to 0.05 was considered to indicate doubtful significance, while a value for P of more than 0.05 was considered to indicate a difference without significance.

TABLE 1.—*Effect of Subcutaneous Steroid Administration on Organ Weight, Proteinuria, and Blood Pressure*

Sex	No.	Steroid	Proteinuria *		Organ Weight Increase; Percentage					Blood Pressure †			Severity Point Rating
			1 Wk.	4 Wk.	Kidney	Adrenal	Heart	Liver	Gonad	Start	4 Wk.	Increase	
Male	18	Control	114	55	19	4	8	32	25	131	145	14	5.5
Male	10	DCA	105	81	30	—18	30	14	20	142	162	20	8.7
Male	15	E ‡	145	95	41	—38	35	37	65	130	149	19	9.4
Male	7	F §	222	161	45	—50	30	37	60	121	159	38	11.0
Female	16	Control	97	96	24	— 4	2	—	31 ‖	126	128	2	5.7
Female	8	E ‡	140	69	24	—54	28	—	41 ‖	122	157	35	8.6

* Milligrams per 100 gm. of body weight per 24 hours.
† Millimeters of Hg.
‡ Cortisone acetate.
§ Hydrocortisone acetate.
‖ Milligrams per 100 gm. of body weight.

TABLE 2.—*Effect of Subcutaneous Steroid Administration on Serum Constituents, Body Weight, and Drawn Blood Volume*

Sex	Steroid	Serum Concentration				Total Protein; %	Final Body Wt.†	Drawn Blood Volume ‡
		Urea *	Creatinine *	Total Cholesterol *	Total Lipoid *			
Male	Control	58	1.14	53	220	5.18	212	3.45
Male	DCA	36	0.75	53	238	5.60	188	3.61
Male	E §	40	0.76	82	374	5.19	136	3.82
Male	F ‖	29	0.53	114	551	5.27	139	3.60
Female	Control	31	0.60	68	328	5.61	176	4.08
Female	E §	26	0.62	87	417	5.94	121	4.18

* Milligrams per 100 cc.
† In grams.
‡ Milligrams per 100 gm. of body weight.
§ Cortisone acetate.
‖ Hydrocortisone acetate.

TABLE 3.—*Effect of Subcutaneous Steroid Administration on Renal Clearance Measurements*

Sex	Steroid	Endogenous Urea Clearance		Endogenous Creatinine Chromogen Clearance	
Male	Control	0.326 *	0.407 †	0.237 *	0.296 †
Male	DCA	0.376 *	0.415 †	0.297 *	0.328 †
Male	E ‡	0.500 *	0.471 †	0.278 *	0.263 †
Male	F §	0.717 *	0.682 †	0.371 *	0.353 †
Female	Control	0.527 *	0.943 †	0.400 *	0.488 †
Female	E ‡	0.718 *	0.760 †	0.337 *	0.354 †

* Milliliters per minute per 100 gm. of body weight.
† Milliliters per minute per gram of kidney weight.
‡ Cortisone acetate.
§ Hydrocortisone acetate.

there were no significant alterations in the urea and endogenous creatinine chromogen clearances, when considered either in relation to body weight or to kidney weight (Table 3).

Cortisone and Hydrocortisone: The effects of cortisone and hydrocortisone administration were very similar, but, as would be expected, the effects of hydrocortisone were more pronounced in most respects, in spite of the slightly smaller dose. The proteinuria was more pronounced than in the control group, both at one week and at four weeks (Chart 2), both during the phase of maximal damage and during the reparative phase, as discussed above.

The point ratings of severity were considerably higher after cortisone and hydrocortisone than in the control group (Table 1). There was a gross increase in the relative size of the kidney, but the microscopic changes were merely those previously described as a concomitant of spontaneous variability in severity of the lesions [7]; this circumstance suggested that administration of these steroids made the lesions more severe but did not produce a significant qualitative alteration in the micro-

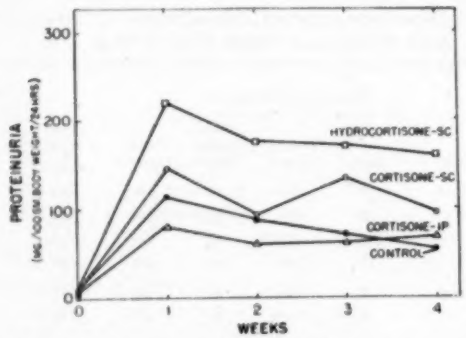

Chart 2.—Effect of subcutaneous cortisone and hydrocortisone and intraperitoneal cortisone on proteinuria of NTG nephritis in the adult male rat.

scopic anatomy. Thus, it is of interest to note that nephritis of the same severity, with approximately the same morphologic characteristics, may be produced by alternative procedures in the male; (1) administration of a given dose of NTG alone, (2) by administration of a smaller dose of NTG followed by daily subcutaneous administration of cortisone or hydrocortisone.

Both steroids produced an increase in the relative cardiac size, but the blood pressure elevation was much greater with hydrocortisone than with cortisone or DCA. In this connection it is of interest to mention that Haynes, Forsham, and Hume found an increase in plasma hypertensinogen after cortisone administration in the dog.[25]

As expected,[26] both cortisone and hydrocortisone diminished the total body growth rate, and the final body weight in each group was only 64% of the control value. The relative liver weight was not significantly affected. The relative adrenal gland weight was greatly diminished, and, in the case of hydrocortisone, dropped to 50% of the predicted weight. There was a moderately pronounced increase in the relative testis weight. Previous reports concerning the effect of cortisone on testis weight are in apparent conflict. Antopol,[26] and Greenspan and co-workers [27]

reported that cortisone administration reduced testis weight, while Winter and co-workers [28] reported no effect. However, in these reports the testis weights are compared without correction for the wide disparity in body weight between the control animals and those given cortisone. Migeon [29] found that the relative testis weight was increased by cortisone administration. Our observations confirm those of Migeon, and it seems clear that, at the least, testis growth is not inhibited by cortisone or hydrocortisone administration to the same degree as total body growth.

Several explanations can be advanced for the increase in relative testis weight after cortisone or hydrocortisone administration, and these explanations may operate as alternates or by addition. It is known that cortin † and cortisone [32] administration produce atrophy of the adrenal glands through inhibition of endogenous corticotropin production. It may be suggested that when corticotropin production is inhibited, the reactions of protein synthesis within the pituitary gland may be altered so as to increase the production of gonadotropin. Another factor might be found in the biological antagonism which has been shown with respect to testosterone

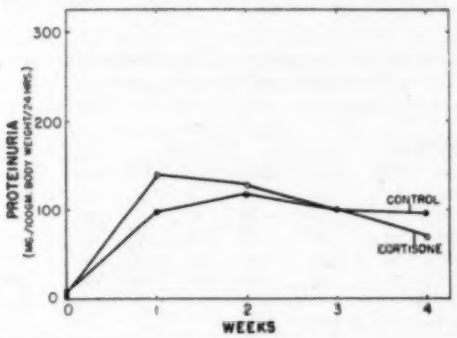

Chart 3.—Effect of subcutaneous cortisone on proteinuria of NTG nephritis in the adult female rat.

and cortisone [33]; an increase in the testis weight might follow an increased requirement for male hormone as the result of such antagonism. Still a third factor might be found in the diminished activity of adrenal androgen production,‡ associated with the adrenal gland atrophy that is induced by cortisone administration, followed by compensatory testis hypertrophy.

Both steroids produced a rise in the serum total cholesterol and serum total lipid concentrations, but, unlike the rise that accompanied an increase in the dose of NTG,[8] there was no appreciable change in the cholesterol/total lipid ratio (Table 2). The serum urea and creatinine chromogen concentrations were lower after administration of cortisone or hydrocortisone than in the control group. After hydrocortisone, the serum urea concentration was only 50% of the control value. No effect was noted in the serum total protein concentrations. The drawn blood volume, which has been shown to give an estimate of the total blood volume,[36] did not vary significantly in these experiments, and it is thus rendered unlikely that concentration changes in chemical constituents of the blood were affected by major blood volume changes.

† References 30 and 31.

‡ References 34 and 35.

With both steroids there was a large increase in the endogenous urea clearance and a less striking rise in the endogenous creatinine chromogen clearance, when calculated relative to the body weight. The urea clearance was also increased relative to the kidney weight, but this was not the case for the creatinine clearance. It may be speculated that, if the renal tubules play a more important role in the excretion of urea than they do in the excretion of creatinine, then perhaps cortisone or hydrocortisone administration increased the excreted fraction of filtered urea by an effect upon tubular function.

Effects of Subcutaneous Cortisone Administration in the Female.—The effects of cortisone given subcutaneously in the female rat after NTG administration were similar to the effects in the male rat in many respects, but dissimilar in effects that concerned the kidney and its functions (Tables 1, 2, and 3). Thus, the effect upon proteinuria was of doubtful significance (Chart 3). No effect was noted on renal size, and no significant effect was noted upon the endogenous clearances of urea and creatinine chromogen. These results are consistent with the sex differences in response to cortisone reported by Wragg and Speirs.[27] There was a moderate, but

TABLE 4.—*Comparison of Subcutaneous and Intraperitoneal Steroid Administration Effects on Organ Weight, Proteinuria, and Blood Pressure*

			Proteinuria *		Organ Weight Increase; Percentage					Blood Pressure †			Severity Point Rating
Sex	No.	Steroid	1 Wk.	4 Wk.	Kidney	Adrenal	Heart	Liver	Gonad	Start	4 Wk.	Increase	
Male	18	Control	114	55	19	4	8	32	25	131	145	14	5.5
Male	10	DCA-SC ‡	105	81	30	−18	30	14	20	142	162	20	8.7
Male	6	DCA-IP §	102	196	84	18	8	32	22	130	146	16	7.3
Male	15	E-SC ‖	145	96	41	−38	35	37	66	130	149	19	9.4
Male	6	E-IP ¶	92	71	13	4	−1	6	18	143	174	31	4.1

* Milligrams per 100 gm. of body weight per 24 hours.
† Millimeters of Hg.
‡ Subcutaneous administration.
§ Intraperitoneal administration.
‖ Subcutaneous administration of cortisone acetate.
¶ Intraperitoneal administration of cortisone acetate.

significant, increase of relative ovary size with cortisone administration. In view of the report that estrogens are antagonistic to cortisone,[28] and in view of the report that estrogens have been isolated from the adrenal glands,[24] the same explanations which were suggested to explain an increase in relative testis size may also be suggested to explain the increase in relative ovary size.

Effects of Intraperitoneal Steroid Administration in the Male.—The effects of DCA given subcutaneously and intraperitoneally are compared in Tables 4 and 5. With intraperitoneal administration, the severity of the renal lesion was increased, with a pronounced increase in the degree of proteinuria (Chart 1) and a parallel increase in cholesteremia and lipemia. However, intraperitoneal DCA did not increase the relative heart size or diminish the relative adrenal gland size so much as the same amount of DCA given subcutaneously; in fact, in this regard there was no difference from the control group.

Intraperitoneal cortisone did not significantly affect the nephritis in any way. The results in this group were closely similar to those in the control group in all respects (Chart 2).

The difference between effects produced by intraperitoneal and subcutaneous injection of adrenal steroids could be the result of several factors. There might be a difference in the rates of absorption, the rates of destruction, and the rates of excretion. There might be differences in the metabolic products, since the subcutaneously injected material would first reach the systemic circulation, while the intraperitoneally injected material would, in large part, first reach the hepatic portal system.

Absorption from the peritoneal cavity is very rapid in the rat. As early as four hours after the intraperitoneal injection of cortisone, no trace of the injected crystals could be discerned in the cavity. After four weeks of daily intraperitoneal injection, no trace of the crystals could be found. On the contrary, after subcutaneous injection, crystals could always be found at the site of injection 48 hours later, and often even 96 hours later.

Although cortisone is inactivated by liver slices in vitro,[39] the experiment cited showed 70% inactivation after three hours' incubation. This is not similar to the

TABLE 5.—*Comparison of Subcutaneous and Intraperitoneal Steroid Administration Effects on Serum Constituents and Body Weight*

		Serum Concentration					
Sex	Steroid	Urea *	Creatinine *	Total Cholesterol *	Total Lipoid *	Total Protein; %	Final Body Wt.†
Male	Control	58	1.14	53	220	5.18	212
Male	DCA-SC ‡	36	0.75	53	238	5.60	188
Male	DCA-IP §	38	0.80	68	341	4.78	200
Male	E-SC ‖	40	0.76	82	374	5.19	136
Male	E-IP ¶	36	0.80	43	266	5.76	211

* Milligrams per 100 cc.
† In grams.
‡ Subcutaneous administration.
§ Intraperitoneal administration.
‖ Subcutaneous administration of cortisone acetate.
¶ Intraperitoneal administration of cortisone acetate.

situation in vivo, and there is evidence to support the view that absorption through the hepatic portal circulation does not materially diminish the effectiveness of cortisone.[40] On the other hand, cortisone is known to disappear rapidly from the systemic circulation.[41] With these observations in mind, it would seem likely that subcutaneous administration resulted in a low, but constantly present level of cortisone in the systemic circulation. Conversely, it would seem likely that intraperitoneal administration resulted in a rapidly attained, high level of cortisone in the systemic circulation, with a rapid subsequent fall in the level, so that the animals were actually under the influence of cortisone during only a small part of the 24-hour period between injections. Our results are consistent with those observed by Greenspan, Gifford, and Deming,[27] who also found that cortisone had little metabolic effect in the rat after intraperitoneal administration.

Hechter and others have demonstrated biosynthetic relationships between various adrenal steroids,[42] with rapid interconversion during perfusion of the liver.[43] Hechter warns that his experiments cannot be assumed to apply without reservations in vivo. However, the increased effect upon the kidney, noted when DCA is administered intraperitoneally rather than subcutaneously, may result from conversion to other

compounds, such as cortisone, in passage through the liver. It should be noted that intraperitoneal administration of DCA did not duplicate perfectly the subcutaneous administration of cortisone, since adrenal atrophy, and cardiac and testicular relative hypertrophy did not occur.

Effects of Adrenalectomy in the Male.—The results of adrenalectomy, compared with sham operation, after NTG administration are given in Table 6. After adrenalectomy, the proteinuria was greatly diminished. The apparent inhibition of blood pressure elevation was not statistically significant. The interval after NTG administration was too short for evaluation of blood pressure changes. It has been shown by Knowlton and co-workers [44] that hypertension will develop after adrenalectomy in rats with NTG nephritis, but this is a late development, after several weeks, and cannot be compared with the conditions of the experiment reported here. It should also be noted that the adrenalectomy experiment is not directly comparable with the steroid administration experiments in this study, as a different time interval and a different lot of NTG were used.

TABLE 6.—*Effect of Adrenalectomy on NTG Nephritis in the Male Rat*

			Organ Weight Increase; Per Cent					Blood Pressure†			Serum Concentration				
Operation	No.	Proteinuria 9 Days*	Kidney	Adrenal	Heart	Liver	Gonad	Start	9 Days	Final Body Wt.‡	Urea§	Creatinine§	Cholesterol§	Lipoids§	Protein; %§
Sham-Adrenalectomy	12	146	6	10	9	12	27	129	139	164	27	0.66	69	280	4.76
Adrenalectomy	6	65	17	..	7	4	24	122	126	145	52	0.68	70	246	4.45

* Milligrams per 100 gm. of body weight per 24 hours.
† Millimeters of Hg.
‡ In grams.
§ Milligrams per 100 cc.

No significant effects upon organ size were noted during the short period of this experiment. There was a significant increase in the serum urea concentration after adrenalectomy; this may be the result of diminished anabolism or increased protein catabolism, since the animals failed to gain weight, while the control group gained 14 gm. during the nine-day period of the experiment.

SUMMARY AND CONCLUSIONS

The effect of adrenal steroid administration upon NTG nephritis in the rat is dependent upon the sex of the animals and the route of steroid administration. Cortisone and hydrocortisone given subcutaneously in the male increased the severity of the nephritis by all criteria, both during the phase of injury and during the phase of repair. Cortisone given intraperitoneally in the male had no effect. Cortisone given subcutaneously in the female had little effect. DCA given subcutaneously in the male produced a slight increase in proteinuria and a more pronounced increase in blood pressure elevation. DCA given intraperitoneally in the male had effects which simulated incompletely those of cortisone given subcutaneously. Adrenalectomy in the male after NTG administration greatly reduced the rate of protein excretion.

Ruth T. Lackey and Jay Banovitz gave technical assistance.

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HEPARIN IN EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN THE RABBIT

I. The Effect of Heparin on the Serum Lipids, and Development of Atherosclerosis

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IT WAS NOTED in 1941 by Chargaff, Ziff, and Moore [1] that heparin had profound effects on the globulin of human serum. The sharp peak which normally accompanies the beta-globulin boundary in the electrophoretic pattern, and which is due to lipids carried by the beta-globulins, was dispersed by heparin. In further studies on naturally occurring and synthetic lipoproteins, Chargaff and his associates [2] found (*a*) that heparin disrupted some lipoproteins, such as the thromboplastic lipoprotein from lung, with the liberation of the combined lipids and formation of a protein-heparin complex, or (*b*) gave rise to heparin-lipoprotein compounds without losing bound lipids, with other lipoproteins, such as lipovitellin from egg yolk. Heparin was therefore shown to possess the property of altering the characteristics of naturally occurring lipoproteins.

More recently, the application of ultracentrifugal methods has extended these observations. Graham and co-workers [3] observed significant changes in the distribution of lipoproteins in normal human serum and in the serum of cholesterol-fed rabbits following the intravenous injection of heparin. An immediate response was observed within five minutes, consisting of a decrease in lipoproteins of the highest S_f classes, with coincident increases in those of the lower S_f classes, the maximum effect being observed in about three hours. The pattern reverted to normal in 24 hours. Continued administration of heparin to cholesterol-fed rabbits inhibited the development of S_f 10-50 lipoprotein macromolecules and, in a short-term experiment, appeared to protect the animals against the development of atherosclerotic lesions. Similar results have been reported by Constantinides and co-workers [15] in a small series of 11 animals. Heparin (10 mg. twice a day) was given subcutaneously. The animals were not litter mates and were not pair-fed, and gross grading only was employed. There was no real difference in the serum cholesterol between treated and untreated animals.

Heparin has also been shown to influence the translucence of serum after fatty meals.*

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* References 4 to 7, 9, and 10.

Anfinsen [8] has investigated the nature of "clearing factor" produced by the intravenous injection of heparin. He has located this active principle in the globulin fraction of the serum proteins and has found that it contains high levels of bound heparin. It is of interest that Anfinsen and co-workers [8] found that the rabbit produced very little clearing factor after injection of heparin. We have been able to confirm this observation.† Gofman, in a private communication to Anfinsen, suggested that this was due to a difference in strains of rabbits used.

Because of the striking effects of heparin on plasma lipoproteins and its reported ability to inhibit atherosclerosis in cholesterol-fed rabbits, the following experiments were undertaken.

MATERIALS AND METHODS

Two series of experiments were done. In the preliminary experiment (*A*) 39 unselected young New Zealand white rabbits 3 to 6 months of age were separated into two groups matched approximately as to age, sex, and weight. In the second experiment, (*B*) 30 pedigreed pairs of littermates, each of the same sex, were used, in an attempt to control the variable response of individual rabbits to cholesterol feeding.

Procedure.—In each series, all the animals received the cholesterol diet for a 12-week period, and the experimental animals received daily intramuscular injections of herapin. The controls were given a placebo injection. Animals were bled from the ear vein at regular intervals for lipid determinations, which were carried out by standard techniques used in our laboratory.‡ At autopsy the severity of aortic atherosclerosis was graded grossly, on a scale of 0 to 4+, and a sketch made of the lesions. On this grading scale, 0 represents either no macroscopically visible lesions, or only very minute ones and is arbitrarily considered to represent an involvement not exceeding 2% of the total surface of the aortic intima with atherosclerotic lesions. Grade 1 represents an average involvement of about 10%, Grade 2 of about 20%, Grade 3 of about 40%, and Grade 4 of 80%, of the intimal surface by atherosclerotic lesions. The means determined from these grades are therefore not strictly arithmetic. In our hands, this method of grading yields fairly consistent results, but we have found that it correlates only roughly with determinations of the actual cholesterol content per aorta. The latter is an objective determination and may be preferable as a method of grading. The intimal and inner medial layers of the aorta were dissected from the adventitia, stored in alcohol, and subsequently extracted in a continuous extraction apparatus for eight hours. The content of cholesterol was determined from this extract. The results are expressed as content of lipid per aorta.

In the preliminary experiment each 100 gm. of the diet contained 1 gm. of cholesterol and 8 cc. of corn (Mazola) oil mixed with the rabbit pellets (Purina). In the second study, 0.75 gm. of dry cholesterol was dissolved in ether and sprinkled on 100 gm. of the pellets. The rabbits were offered 100 gm. of fresh food daily. Food-intake records were kept and the animals were pair-fed.

Heparin§ was dissolved in a special menstruum for intramuscular injection. The composition per cubic centimeter was as follows: sodium heparin 200 mg., gelatin 180 mg., dextrose 80 mg., and water. In the first experiment 50 mg. of heparin was injected daily intramuscularly into each rabbit of the experimental group. In the second experiment 20 mg. of heparin per kilogram of body weight was injected daily into one of each pair of littermates while the other received a similar injection of gelatin-dextrose menstruum daily. Preliminary tests had shown that this dose of heparin gave a prolongation of the clotting-time for a period of from 8½ to 20 hours.

† Unpublished data.

‡ References 13 and 14.

§ Supplied by the Upjohn Company, Kalamazoo, Mich.

RESULTS

Experiment A.—Eight of the 39 animals died prior to the conclusion of the experiment, and, of these, 6 died prior to the eighth week and were excluded from further consideration. In four animals hemorrhage into the tissues was the major factor resulting in death.

Morphological (Gross) Grading: The distribution of the severity of aortic atherosclerosis as judged by gross grading is shown in Table 1. It is at once apparent that the heparin-treated animals had a lower degree of atherosclerosis than did the controls. The arithmetical means of gross grades of atherosclerosis in the experimental and control groups were 2.5 and 3.59 respectively. The probability of obtaining this result is less than 0.01, and the difference therefore a significant one.

TABLE 1.—*Summary of Grading Of Aortas: Experiment A*

Controls				Experimentals			
		Cholesterol Mg./Aorta				Cholesterol Mg./Aorta	
Rabbit No.	Gross Grade	Free	Total	Rabbit No.	Gross Grade	Free	Total
V-56	4	9.84	28.53	S-63	4	6.25	17.60
V-2	4	14.17	50.71	V-9	4	7.10	23.45
V-3	4	14.69	40.47	V-54	4	4.27	13.46
V-5	4	9.76	28.15	V-48	4	6.76	16.11
U-79	4	6.83	26.59	S-62	3	5.12	13.71
U-99	4	....	31.13	U-87	3	2.80	5.35
V-6	4	8.87	28.53	V-11	3	3.25	10.15
V-10	4	8.63	23.98	V-52	3	3.59	10.43
S-58	4	13.66	34.17	V-53	3	4.09	11.03
U-98	4	11.25	30.20	U-98	2	2.87	6.76
U-88	4	6.48	20.62	U-76	2	1.35	3.20
U-86	3	11.47	13.81	U-80	1	1.30	2.50
V-7	3	5.15	15.10	U-85	1	1.25	2.60
U-82	3	6.48	18.34	V-8	1	2.55	5.35
U-89	3	3.10	8.29	V-50	1	0.68	2.17
V-57	1	1.06	0.95	V-49	1	0.80	2.17
* V-55	4	....	.....	.....	..	....	.....
Mean.....	3.49 †	8.76	24.96		2.5 †	3.35	9.12
S.D. of mean	±0.79	±3.99	±12.21		±1.21	±2.11	±6.46
S.E. of mean	±0.20	±1.07	± 3.16		±0.31	±0.55	±1.67
P.....	<0.01	<0.001	< 0.001				

* Extract lost.
† Not linear.

Cholesterol Content of Aortas: The results of gross grading were consistent with the determinations of cholesterol content of the aortas, as seen in Table 1. The means of total cholesterol content were 9.12 mg. for the heparin-treated animals and 24.96 mg. for the controls. The difference is highly significant statistically.

Serum Lipids: On the whole, the heparin-treated animals tended to have lower serum lipid values than the controls, but the differences were not significant. Actual values are shown in Table 2.

Experiment B.—Twenty rabbits died or were killed prior to the eighth experimental week, and they have not been included in the final evaluation. Two additional pairs were broken up early in the course of the experiment, and the survivors could not be used. Eighteen pairs survived more than eight weeks and form the substance of this report.

Morphological (Gross) Grading: There were five pairs of rabbits in which the lesions were of comparable severity, and on the whole the lesions were minimal, the largest difference being 1 grade. There were two pairs of rabbits in which the heparin-treated animal had severer lesions than the control littermate. This group included

TABLE 2.—*Summary Of Serum Lipid Values: Mean Values Experiment A*

	Group	Experimental Weeks 0	3	6	9	12
Cholesterol, total, mg./100 cc.....	Heparin-treated	44.38	490.3	2,973.6	1,653.0	1,481.8
	Control	52.65	2,021.3 *	2,308.6	2,094.1	2,111.3
Cholesterol, ester, mg./100 cc.....	Heparin-treated	34.38	376.0	2,105.2	1,233.7	1,052.2
	Control	42.17	1,617.5 *	1,703.0	1,569.7	1,458.8
Cholesterol, free, mg./100 cc.....	Heparin-treated	9.6	114.5	868.4	419.3	429.7
	Control	11.5	403.8 *	605.6	524.4	652.4
Lipid phosphorus, mg./100 cc.....	Heparin-treated	4.8	13.3	36.3	20.9	24.5
	Control	4.2	23.2 *	28.1	24.7	30.2
Total fatty acids, mEq./l.....	Heparin-treated	7.9	22.9	72.1	25.0	37.9
	Control	6.6	58.8 *	60.4	52.7	66.3
Fatty acids of neutral fat, mEq./l.....	Heparin-treated	4.8	6.9	21.4	10.83	15.8
	Control	3.7	3.7	3.7	12.5	20.9

* Statistically significant $P = 0.001$.

TABLE 3.—*Summary of Grading Of Aortas: Experiment B*

Controls				Experimentals			
		Cholesterol Mg./Aorta				Cholesterol Mg./Aorta	
Rabbit No.	Gross Grade	Free	Total	Rabbit No.	Gross Grade	Free	Total
X-17	4	10.38	25.53	X-18	2	4.44	10.79
W-89	4	11.31	29.40	W-90	1	1.94	4.39
W-69	4	9.84	33.65	W-70	2	3.06	7.28
X-9	3	6.48	17.72	X-10	0	0.65	1.15
X-23	3	6.43	21.36	X-24	1	0.44	1.67
W-97	3	4.48	10.91	W-98	0	0.76	1.04
X-13	2	3.91	10.91	X-14	2	2.42	5.73
X-19	3	5.27	11.83	X-20	1	1.02	1.51
X-15	1	1.37	5.63	X-16	1	0.76	4.96
X-77	1	0.79	1.07	W-78	0	1.15	1.85
W-99	1	1.07	1.34	W-100	3	4.85	12.39
W-73	1	1.02	1.05	W-74	0	0.96	1.25
W-87	1	1.28	2.34	W-88	0	1.47	3.04
W-91	1	1.45	2.51	W-92	1	1.37	3.18
X-21	0	0.26	0.86	X-22	0	0.94	1.21
W-93	0	0.94	1.50	W-94	1	1.68	3.94
W-85	0	1.04	1.79	W-86	0	0.97	1.46
W-95	0	0.91	0.74	W-96	0	1.01	1.84
Mean.....	1.72	3.79	10.00		0.83	1.66	3.82
S.D. of mean	±1.45	±3.69	±10.98		±0.93	±1.27	±3.36
S.E. of mean	±0.35	±0.90	± 2.67		±0.23	±0.31	±0.82
P.....	<0.02	<0.02	< 0.02				

rabbit W-100, which had lesions of Grade 3 severity, the severest in the heparin-treated group. There were 11 pairs of rabbits in which the control animals had more marked lesions than the heparin-treated ones. In two pairs the difference in grading was very marked (3 grades), in four pairs it was marked (2 grades) and in five pairs it was slight (less than 1 grade). From Table 3 it is apparent that there is a considerable difference between the treated and untreated groups. The means

of the grades were 1.72 for the control group and 0.88 for the treated animals. The probability of obtaining this result by chance is less than 0.02, and the difference is significant.

Cholesterol Content of Aortas: The cholesterol content of the aortas was distinctly less than in the preliminary experiment. This was not surprising in view of the fact that the daily dose of cholesterol was smaller and both the serum lipid levels and gross lesions were of less magnitude.

In the control group the values for total cholesterol content ranged between 0.74 and 33.65 mg. and the mean was 10.0 mg. In the experimental group the range of cholesterol content was from 1.04 to 12.39 mg. and the mean was 3.82 mg. The difference of the means is statistically significant (Table 3).

Serum Lipids: As in the initial experiment, the heparin-treated animals tended to have lower serum lipid levels, but again the differences were not significant, except for the serum cholesterol values at the termination of the experiment (Table 4).

TABLE 4.—*Summary of Serum Lipid Values: Mean Values, Experiment B*

		Experimental Weeks							
	Group	0	0-1	1-2	2-3	3-4	5	9	13
Cholesterol, total, mg./100 cc.	Heparin-treated	36.6	95.5	299.2	384.9	199.0	433.6	371.6	609.8
	Control	32.9	128.0	315.0	366.9	472.3*	553.6	479.0	1,201.1†
Cholesterol, ester, mg./100 cc.	Heparin-treated	29.4	67.8	210.0	277.4	140.3	319.0	284.2	406.8
	Control	25.5	98.2	210.3	235.4	336.5*	408.4	347.5	880.04†
Cholesterol, free, mg./100 cc.	Heparin-treated	7.2	27.8	89.2	107.4	58.7	114.6	87.4	203.0
	Control	7.4	34.8	104.8	131.4	135.8	145.2	122.5	321.0*
Lipid phosphorus, mg./100 cc.	Heparin-treated	4.0	8.0	10.9	11.1	8.1	11.9	14.9	20.8
	Control	4.0	7.3	8.6	13.5	10.8	13.1	15.1	24.3
Total fatty acids, mEq./l.	Heparin-treated	7.2	10.5	15.0	12.5	12.7	17.7	22.2	31.8
	Control	7.6	10.0	11.5	22.1	18.3	18.9	22.3	40.3

* $P = < 0.2$.
† $P = < 0.05$.

Summary of the Results in Littermate Pairs.—The littermate pairs have been divided into three groups on the basis of cholesterol content of the aortas.

Group I: In this group there was no significant difference in the aortic cholesterol content in members of the pairs. Of the nine pairs of animals in Group I, the controls gained more weight than the heparin-treated animals in every instance, despite pair-feeding and relatively similar intakes of cholesterol. The serum lipid showed marked swings in nearly every pair, reflecting the erratic food-intake of the animals. The serum cholesterol values were not particularly high in any of the animals and did not in any case exceed 1,000 mg. per 100 cc. In three pairs (W-95-96; W-77-78; W-93-94), the lipid levels of the heparin-treated member of the pair were higher than in the control rabbit. In the other six pairs, there were no obvious differences between the lipid levels; in all cases, however, the final cholesterol values were higher in the control animals. Atherosclerosis was slight in this group, and none of the animals had lesions of a severity greater than Grade 1. In only one instance (X-15-16) was the aorta cholesterol content greater than 5 mg. We may conclude, therefore, that these nine pairs were relatively resistant to the action of ingested cholesterol. The blood cholesterol levels did not rise adequately and intimal lesions were slight.

Group II: In this group the heparin-treated animal had higher aortic cholesterol content than the control. It included only one pair, W-99-100. Food intake was excellent; body weights were about the same in the two animals. Serum lipids were consistently higher in the heparin-treated member of the pair. At five weeks, the total serum cholesterol was 700 mg. per 100 cc. in the heparin-treated animal and only 300 mg. per 100 cc. in the control. At 9 and 13 weeks, the values were 700 and 1,100 mg. per 100 cc. for the heparin-treated animal and 100 and 200 mg. per 100 cc. for the control. The heparin-treated animal had much severer gross lesions of the aorta and higher aortic cholesterol-content (nearly 10 times as much) as the control. This was the only pair in which the experimental animal had significantly more cholesterol in its aorta than the control.

Group III: This group consisted of eight pairs, and in every pair the control animal had more cholesterol in its aorta than did the heparin-treated animal. The controls gained more weight than did the heparin-treated animals. Food intake was excellent in three pairs and erratic in the remainder. In every instance, the serum lipids were higher in the control animals than in the heparin-treated animals. The highest serum cholesterol value achieved by any of the animals was about 2600 mg. per 100 cc. (X-17), but all the control animals exceeded 1,000 mg. per 100 cc. With the exception of two pairs (W-89-90 and X-17-18), the differences in serum lipid levels between members of the pairs were large. In the latter two pairs, despite negligible differences in serum lipid levels, there were marked differences in severity of the aortic lesions. It would appear that, in general, the blood cholesterol of the heparin-treated animals was depressed, while the serum lipid phosphorus level was only slightly reduced, resulting in a drop of the cholesterol-phosphorus ratio. It is doubtful, however, that the differences in aortic atherosclerosis can be ascribed to the serum lipid changes.

COMMENT

In both the first (*A*) and the second (*B*) experiment there were significant differences in the degree of atherosclerosis between the control and heparin-treated animals, as judged by gross grading, and this was confirmed by the chemical estimation of cholesterol content of the aortas. The first experiment (*A*) was a pilot experiment, the results of which could not be regarded as unequivocal. In the second experiment (*B*) special measures were taken to control both animals and diet. Littermate pairs of the same sex were used. In our experience this reduces the errors introduced by the well-known variability of response of rabbits to cholesterol feeding, a vitally important factor when small numbers of animals are used. Further, the concentration of dietary cholesterol was reduced from 1 to 0.75% and oil was omitted in an attempt to prevent a masking of a possible effect of heparin action by the excessive cholesteremia and atherogenesis which customarily result from the cholesterol and oil diet. By means of pair-feeding it was possible to keep the cholesterol intake of littermates practically the same. Nevertheless, the heparin-treated animals failed to gain as much weight as the controls, the mean difference in weight being 110 gm. Firstbrook[11] has concluded that relative undernutrition may lead to partial inhibition of atherosclerosis, but experiments in this laboratory have shown that undernourished animals develop as much atherosclerosis as controls fed the full diet.[12]

In the second experiment, adequate control was maintained, and the results may be considered as unequivocal. In 11 pairs, the control animal had greater lesions, judged grossly, than the heparin-treated animal, and the trend was reversed in only two pairs. This was confirmed by the cholesterol content of the aortas; in eight pairs the difference exceeded 5 mg. of cholesterol and was far greater in many other instances (Table 3).

The results of serum lipid analysis failed to demonstrate any consistent difference which might be attributable to heparin administration. In experiment (*A*) a significant lowering of all the serum lipids was noticeable at the third week, but this was not confirmed in experiment (*B*), where special attention was directed to serum lipid levels in the first few weeks of cholesterol feeding.

In the eight pairs of animals where the difference in aortic cholesterol content exceeded 5 mg. per aorta, inspection of serum lipid levels revealed that the controls had higher lipid levels than the heparin-treated in six pairs and there was no difference in two pairs. This, plus the fact that no real difference in serum lipids was seen on a group basis, suggests that heparin does not exert its effects on atherosclerosis because of quantitative changes in serum lipid levels. Heparin does, however, influence the lipoprotein pattern of the serum, and this may well provide the explanation of its action. The failure of heparin to act in all instances may perhaps be related to the availability of other serum or tissue factors, such as clearing factor precursor, tissue factor, or serum co-protein described by Anfinsen.[8]

SUMMARY

The administration of large doses of heparin (by intramuscular injection) to cholesterol-fed rabbits retards the development of atherosclerotic lesions of the aorta, as judged by gross grading and by chemical determination of the cholesterol content of the aortas.

Heparin has no consistent effect on the content of lipids in the serum of cholesterol-fed rabbits, and it is presumed that it exerts its effect by modifying the serum lipoprotein pattern.

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CONGENITAL ABSENCE OF INTRAHEPATIC PORTAL VENOUS SYSTEM SIMULATING ECK FISTULA

Report of a Case with Necropsy Findings

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CONGENITAL absence of the terminal portion of the portal vein and of its intrahepatic branches is an uncommon finding. It has not been previously described in humans. The only comparable case in the literature is in a dog reported by Hickman, Edwards, and Mann in 1949.[1]

REPORT OF A CASE

The subject of this report was a 5½-months-old white girl, who was born three to four weeks prematurely after an otherwise normal pregnancy. Jaundice was present at birth, and it faded when the infant was one month of age. Slight cyanosis was noted at birth and gradually increased with the appearance of symptoms of wheezing and coughing. The infant began to vomit occasionally at 2 months of age. This continued until the time of admission, but in spite of it she continued to gain weight slowly. At this time the infant was cyanotic and dyspneic but active. Five or six protuberant hemangiomata, 0.3 to 0.9 cm. in diameter, were noted on the trunk, thigh, and upper lip. The lungs were clear to percussion and auscultation. The heart was slightly enlarged bilaterally; a blowing systolic murmur was heard over the cardiac apex with a continuous low-pitched murmur over the entire precordium. The abdomen and the extremities appeared normal. A roentgen study revealed a soft, pulsating density in the mediastinum along the right heart border. There was atelectasis of the right upper lobe with a shift of the trachea and upper esophagus to the right. The electrocardiogram showed an incomplete right bundle branch block with a right ventricular hypertrophy. A right exploratory thoracotomy was performed. The surgeon described "a vascular tumor in the right cardiophrenic angle," but no biopsy was performed. Postoperatively, the respirations remained rapid, the pulse rate ranged between 140 and 190, and the patient was cyanotic when out of oxygen. On the 9th postoperative day, the infant became increasingly cyanotic, her respirations became difficult and noisy, and she died on the 10th postoperative day.

At autopsy, the heart was greatly enlarged (100 gm., normal 29 gm.).* Both ventricles were dilated and hypertrophied (the right and left measuring 1.0 and 1.3 cm. in thickness respectively). The ductus arteriosus and foramen ovale were patent, the ductus measuring 0.3 cm. and the foramen 0.8 cm. in diameter. The enlarged heart, which occupied most of the left pleural cavity, compressed the left lung. There were pulmonary edema and congestion of the viscera. The segment of the portal vein extending from its confluence with the coronary and splenic veins to the usual point of entry to the liver, as well as its entire intrahepatic distribution was absent. (Fig. 1). The usual tributaries of the portal vein drained into a large vessel which took its course directly cephalad along the posterior liver surface; it joined the hepatic vein at the superior liver edge. This vessel and the hepatic vein joined to form the supradiaphragmatic portion of the inferior vena cava which entered the right atrium normally. The subdiaphragmatic portion of the inferior vena cava was formed by the confluence of the right and left

From the Department of Pathology of the Children's Medical Center and the Children's Cancer Research Foundation.

* Normal values from Coppoletta and Wolbach.[2]

common iliac veins approximately at the level of the fifth lumbar vertebra. It was then joined by the renal veins. It passed through the diaphragm on the right lateral aspect of the thoracic vertebrae to join the enlarged azygos vein. This vessel then entered the right atrium in a normal position. The falciform ligament and ligamentum teres were absent. The hepatic artery, normal in structure and caliber, was the only blood vessel entering the liver hilus, accompanying the hepatic bile ducts. The liver itself was relatively small (165 gm., normal 188 gm.) and was abnormally lobulated. The left lobe included approximately two-thirds of the parenchymal substance and was located principally in the middle epigastrium. Several small, irregular, dome-shaped lobules were present on the anterior and posterior surface of the liver. The usual caudate and quadrate lobes were not present. The gall bladder was buried in the substance of the liver. The cystic duct entered the right hepatic duct.

On microscopic examination, all heart sections, but chiefly those from the subendocardial and interventricular septal regions, had numerous small infarcted areas.

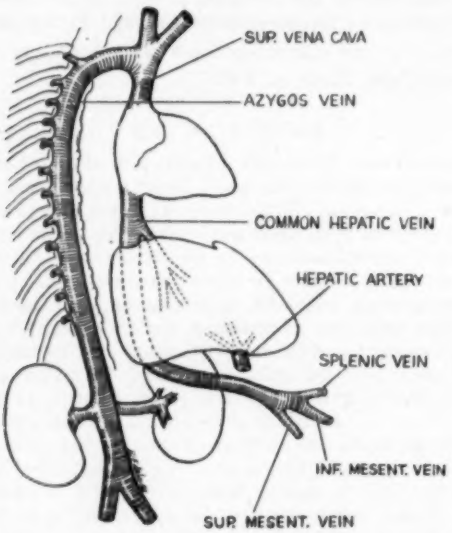

Fig. 1.—A schematic diagram of the abdominal venous drainage.

The muscle fibers here were necrotic and surrounded by a rim of young fibroblasts and occasional lymphocytes (Fig. 2). There were fresh hemorrhages and calcium depositions in some of these areas. The surrounding muscle fibers contained numerous fine glycogen droplets. The preserved muscle fibers were hypertrophic with coarse cross-striations and were separated by edema. A significant feature in the lung sections besides edema, pneumonitis, and marked capillary congestion and atelectasis, was a marked increase in the number of small arteries. They had a thickening of their media, and in some of them there was focal proliferation of their intima. The liver sections showed a distortion of the hepatic architecture with a marked variation in the size of the liver lobules. The periportal areas contained normally formed bile ducts and (Fig. 3) numerous coiled small branches of the hepatic artery. No intrahepatic branches of the portal vein were recognized. The liver cells throughout the sections contained small fat vacuoles. Small areas of

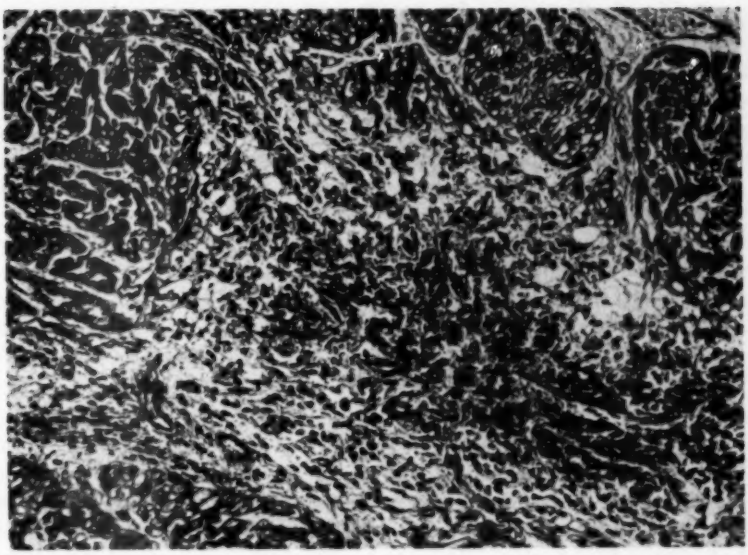

Fig. 2.—Heart; a small area of myocardial infarction surrounded by early fibrosis. Hematoxylin and eosin; × 150.

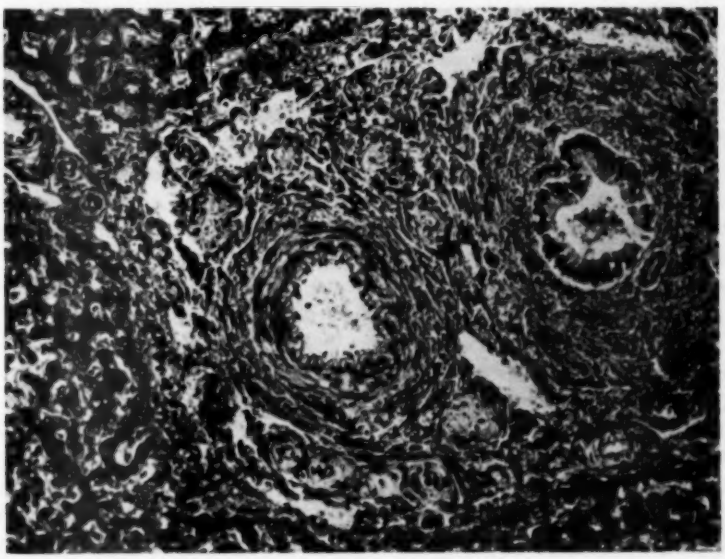

Fig. 3.—Liver, periportal area; a bile duct is seen together with a large artery and several smaller arterioles in cross section. No portal vein branch is seen. Hematoxylin and eosin; × 150.

necrosis were present in some central areas. The sinusoids in the intermediary and peripheral lobular zones were dilated. The microscopic sections of the remaining viscera were not remarkable.

In summary, this case thus presents multiple congenital anomalies consisting of the following: an absence of the intrahepatic distribution of the portal vein; a termination of the aberrant portal vein in the supradiaphragmatic portion of the inferior vena cava joining the hepatic vein; a drainage of the subdiaphragmatic portion of the vena cava into an enlarged azygos vein; an abnormal lobulation of the liver with an absent ligamentum teres; an intrahepatic position of the gall bladder, with the cystic duct entering the right hepatic duct; a malrotation of the large intestine, and multiple hemangiomata of the lip and skin. There was a cardiomegaly with hypertrophy and dilatation of both ventricles and multiple areas of myocardial infarction. The ductus arteriosus was patent. Signs of cardiac failure were evident in the visceral congestion and edema. Pneumonitis of the right lung and atelectasis of the left lung were present.

COMMENT

The cause of death must be ascribed to cardiac failure in view of the marked cardiac dilatation, the pulmonary and visceral congestion, and the lymph stasis. This resulted from an increased cardiac output which, in turn, resulted in the marked cardiac hypertrophy. There are two possible mechanisms by which this may have been brought about. First, with the widely patent ductus arteriosus, there would be a left-to-right shunt and increased pulmonary pressure. Secondly, with an absence of the intrahepatic circulation of the portal blood, an increased cardiac output might result, as in an arteriovenous shunt, with the failure of passage of blood through portal sinusoids. These two conditions are considered responsible for the marked hypertrophy of both ventricles. Increases in pulmonary pressure may be accompanied in some cases of patent ductus arteriosus by a medial hypertrophy in the small muscular pulmonary arteries.[3]

An increased flow within the hepatic artery circulation might be postulated to provide a substitute blood supply to the hepatic parenchyma, and this increase helps to explain the microscopic changes in the branches of the hepatic artery. The corkscrew pattern of their course simulates that seen in the endometrial arteries of the pregnant uterus and in the region of a corpus luteum of pregnancy where a sudden fall in pressure must be accommodated †—in this case from an arterial pressure to that of the hepatic sinusoids at a volume of flow greatly over normal.

The microscopic changes in the myocardium are considered to be due to hypoxemia resulting from the marked myocardial hypertrophy. Very similar features have been observed by Boemke[6] in a series of congenital heart malformations. According to Boemke, hypoxemia may lead to hyaline thickening of arteriolar and capillary walls and cause a further decrease in oxygen permeability. The oxygen supply thus becomes sufficiently inadequate to cause multiple small myocardial infarctions.

A patent ductus arteriosus alone does not usually cause cardiac failure in this age group and so does not give a sufficient explanation for the degree of circulatory distress. The degree, however, to which the vascular anomaly of the liver takes part in the circulatory dysfunction, is difficult to determine.

† References 4 and 5.

Functionally, this case resembles an Eck fistula. Eck fistula dogs may be maintained in relatively good health for several years (Whipple [7]). As observed repeatedly in man (Simonds [8]), in cases of portal venous obstruction, and in experimental and therapeutic Eck fistula, it is quite possible to maintain life without significant clinical symptoms or pathological change. The absence of the intrahepatic portal distribution in this case did, however, cause alterations in the anatomical structure of the liver. It is, however, unlikely that these structural alterations caused hepatic dysfunction, since the liver cells were normal in appearance.

According to the usual description of the development of the liver, there is proliferation of vascular channels originating from the vitelline veins between the primitive liver cords. The endothelium of the primitive vascular growth finally resolves into a network of sinusoids. This vascular network is believed to stimulate the growth of the liver. In this case, one must assume that the vitelline veins originally did enter the liver but that the connection with the vitelline veins was interrupted. Then the derivatives of the vitelline veins joined the hepatic vein at a later stage of development, without further branching into the liver parenchyma. The umbilical veins failed to enter the liver but apparently passed inferiorly along the abdominal wall to the iliac veins (Bremer ‡). Because of this, no falciform ligament was formed. The abnormal lobulation of the liver may also be the result of this failure in growth stimulation.

During the development of the inferior vena cava in this case, the hepatic segment was apparently not formed, but the upper segment of the right subcardinal vein, which joins the azygos vein, persisted. The systemic vein thus consisted of the lower portion of the inferior vena cava, the remnant of the right subcardinal vein, and the upper portion of the azygos vein.

There is, however, no way to prove these suggested relations, and the true embryological events in this case remain obscure. The other anomalies, the persistent ascending and descending mesocolon, the patent ductus arteriosus, and the multiple hemangiomata are judged to be only associated, on the basis of a general tendency for congenital malformation.

As previously mentioned, the only comparable case is that observed in a dog reported by Hickman, Edwards, and Mann in 1949.[1] They found (1) a continuity of the lower part of the inferior vena cava with the enlarged azygos vein, which entered the superior vena cava just above the right atrium, and (2) an absence of the portal vein and a drainage of the gastrosplenic, pancreatic, and ileocecocolic veins into a short common trunk which joined the azygos vein. Only the hepatic artery and common bile duct were present on the inferior liver surface; both were normal in structure and anatomical arrangements. The hepatic vein entered the right atrium separately as a small vessel. Microscopic examination revealed a normal lobular arrangement; the sinusoids were considerably congested. No anomalies were found in the heart or lung.

A functionally similar condition, however, has been described in man by Abernethy (1793),[9] Lawrence (1814),[10] and Kiernan (1833).[11] Each of these observed a case of the portal vein draining into the inferior vena cava or azygos vein respectively. Abernethy's case was a 10-month-old girl, who had situs inversus of the thoracic organs and a drainage of the lower part of the inferior vena cava into

‡ Bremer, J. L.: Personal communication to the author.

an enlarged azygos vein, where it was joined by the portal vein. Although the hepatic artery was the only vessel entering the liver, no microscopic anomalies of the portal venous system were noted. No other anomalies were found in any organ, and the cause of death remained obscure. Lawrence reported a case which he did not observe himself, but was similar to that described by Abernethy. Kiernan's case was a 13-year-old girl whose accidental death followed a head injury. The portal vein and an accessory splenic vein drained into the inferior vena cava. The course of the inferior vena cava was normal. The hepatic artery was larger than usual, and it was the only vessel entering the liver. Again, no anomalies of the intrahepatic portal system were noted and no other pathological signs were observed.

None of these cases showed any evidence of liver, heart, or pulmonary disease. The main difference between these cases and the case reported here appears to be the absence of the intrahepatic distribution of the portal vein in this case, which seems responsible for the microscopic changes in the liver parenchyma.

An anomalous development of the inferior vena cava in association with a normal portal vein circulation has been described several times (Griffith [12] and Miller [13]). According to Henke and Lubarsch,[14] a participation of the portal vein in arrested development of the inferior vena cava occurs very rarely because of the ontogenetically earlier development of the hepatic circulation.

SUMMARY

A case of congenital absence of the terminal portion of the portal vein and of its intrahepatic distribution has been observed. It was associated with a drainage of the subdiaphragmatic vena cava into the azygos vein, an absence of the ligamentum teres, a malrotation of the large intestine, and a patent ductus arteriosus.

This case thus presents a combination of developmental anomalies, which resulted in death from circulatory failure due to the increased cardiac output. An identical case has not been reported previously in the literature.

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ALTERATION IN GROUND SUBSTANCE IN EXPERIMENTAL NECROTIZING ENDOCARDITIS

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IN THE study of the reaction of tissues to injurious agents the emphasis has been shifted in recent years to the changes taking place in the extracellular matrix and is no longer concentrated almost exclusively on alterations in the cells themselves. This shift has been stimulated, or at least facilitated, by the development of histochemical techniques which demonstrate the components of the ground substance much better than conventional histologic stains do. In fact, as is illustrated in this report, such substances can be identified in areas which when observed only with conventional stains were interpreted simply as representing accumulations of edema fluid. As yet, the histochemical methods yield relatively meager information about the chemical nature of the substances demonstrated. Some interpretations are matters of considerable debate, and some recently held interpretations have been clearly shown to be invalid. Better interpretations await elucidation of the chemical nature of the substances demonstrated and chemical description of the reactions involved in the techniques.

Nevertheless, these methods have been used widely, and interest in alterations in the nature and amount of the ground substance in various conditions grows steadily. Altshuler and Angevine [1] concluded that the essential component of fibrinoid necrosis was a change in the acid mucopolysaccharides of the ground substance. Later the same authors [2] reported finding an increased amount of acid mucopolysaccharide in lesions produced by a wide variety of agents, ranging from anoxia to x-irradiation. Moon and Rinehart [3] report that an acid mucopolysaccharide is the most consistent component of the early lesions of coronary arteriosclerosis. Articles too numerous to cite in detail have described the effects of various hormones and enzymes on the ground substance. At least three recent reviews * and one published conference [7] have emphasized the importance of the role of the ground substance in tissue reactions. There is to be reported here another instance of a lesion, in this case induced by diet and renal insufficiency, in which an alteration in the ground substance appears to play an essential part.

Necrotizing arterial and endocardial lesions have been produced consistently in this laboratory by feeding a high fat diet for eight weeks or longer and then induc-

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* References 4-6.

ing renal insufficiency. The excess dietary fat is in the form of ordinary creamery butter. The renal insufficiency is induced by injections of mercuric chloride or uranium nitrate or by bilateral nephrectomy. The lesions produced are identical, regardless of the means used to cause renal insufficiency. A detailed description of the diet and many other aspects of the experimental production of these lesions can be found in previous articles.†

About 90% of the dogs subjected to the combination of high fat diet and renal insufficiency develop necrotizing lesions of the endocardium of the heart, the aorta, or small arteries of other organs. Approximately 70% of the dogs with such lesions in any organ show a lesion in the endocardium of left auricle just above the posterior cusp of the mitral valve. This single site is the one which has been most consistently involved, and this tissue was studied in groups of control and experimental animals by means of the histochemical techniques described below.

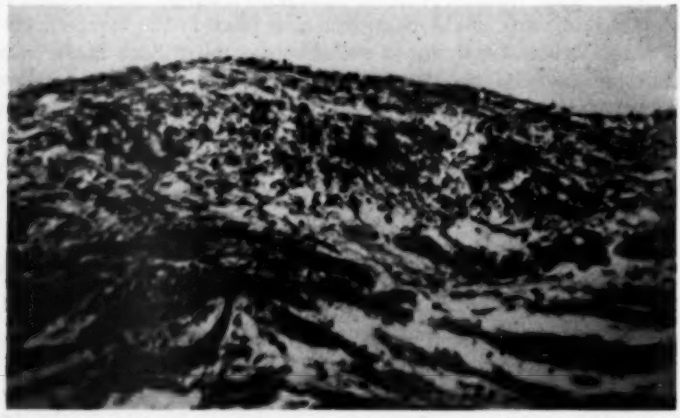

Fig. 1 (Dog 52-19).—Hematoxylin and eosin stain of left auricular endocardium. Eight weeks of high fat diet; nine days after injection of uranium nitrate. Note thick endocardial layer with fragmentation of collagenous and elastic fibers, amorphous granular debris in center, numerous clear areas, and leucocytic infiltration.

The microscopic appearance of these typical endocardial lesions in a relatively early stage is illustrated in Figure 1. The dog was fed the high fat diet for eight weeks and then given a subcutaneous injection of 8 mg. of uranium nitrate per kilogram of body weight. Nine days after injection the blood urea nitrogen had risen to 220 mg. per 100 cc. and the dog was killed and necropsied immediately. On the endocardium of the left auricle above the posterior cusp of the mitral valve was an elevated opaque yellowish granular area. Figure 1 shows in a routine hematoxylin and eosin stain the thickening and cellular infiltration of the endocardium, the swelling and fragmentation of elastic tissue, and particularly the separation of the fibers by large clear areas. The appearance of these lesions is substantially the same whether found in the endocardium of the left auricle, the pulmonary artery, the aorta, or smaller vessels in other parts of the body.

The histochemical techniques employed in these studies included (1) the Hale technique as modified by Rinehart and Abul-Haj,[10] (2) the metachromatic reaction

† References 8 and 9.

of toluidine blue O, and (3) the periodic acid-Schiff (PAS) reaction. These were applied regularly to formalin-fixed paraffin-embedded tissues. A few sections were prepared by freeze-drying, paraffin embedding, and alcoholic fixation of the deparaffinized sections, and these yielded substantially similar results as the formalin-fixed tissue. A few selected sections, both formalin-fixed and frozen-dried, were subjected to digestion by commercial hyaluronidase (Abbott's Hyazyme) for 1 hour at 37 C. and stained by the above methods.

RESULTS

Out of 11 dogs which had been subjected to the usual experimental procedure of high fat diet plus renal insufficiency, 7 had necrotizing lesions of the left auricular endocardium. It was found that the early stages of the lesions such as those illustrated in Figure 1, were associated with heavy deposits of material which (1) reacted positively to the Hale stain, (2) was metachromatic with toluidine blue O,

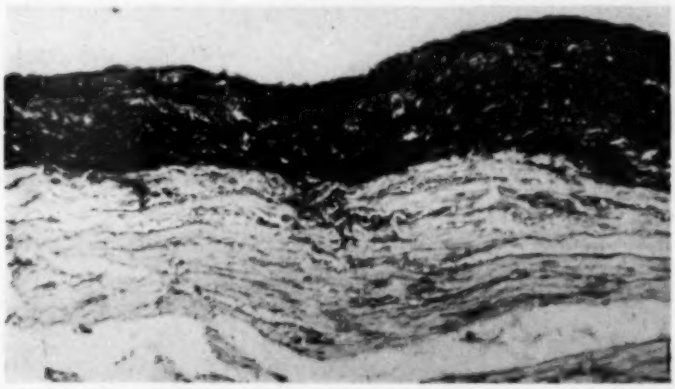

Fig. 2 (Dog 52-19).—Hale stain of left auricular endocardium. Photographed with Wratten F filter (dark red). Cut from same block as section shown in Figure 1. Note intense deposit of blue-staining material (here shown as black) in the thickened endocardium.

and (3) gave only faint coloration with PAS. It is seen in Figure 2 that this material occupies the spaces which are clear in the hematoxylin and eosin stain, and which were formerly interpreted simply as edema. The toluidine blue O metachromasia corresponds closely to the distribution of this blue material but cannot be illustrated well in black and white. Figure 3 shows the PAS stain on a parallel section from the same block. The myocardial glycogen is intensely stained but there is only a small amount of PAS-positive material in the center of the lesion. The glycogen is removed by salivary digestion but the material in the lesion is not. The large deposits of Hale-positive metachromatic material react only slightly, if at all, to the PAS stain. None of it is removed by hyaluronidase as used.

In lesions where necrosis was further advanced, with complete loss of collagenous and elastic fibers and accumulation of cellular exudate and necrotic debris, the Hale-positive metachromatic material disappeared and somewhat more PAS-positive material appeared.

In an attempt to better define the factors involved in the production of these changes, several series of control animals were examined in a similar manner. The

first of these was a group of 22 supposedly normal dogs recently received in the animal room and kept on the regular kennel diet for varying periods. A surprising number of these dogs showed in the section taken from the left auricular endocardium above the posterior cusp of the mitral valve a characteristic change illustrated

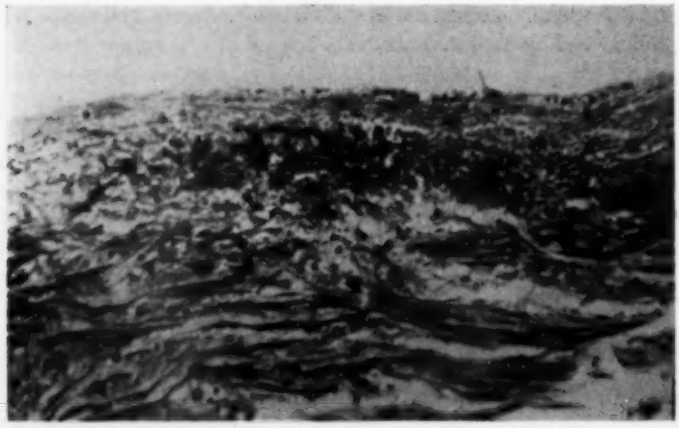

Fig. 3 (Dog 52-19).—PAS stain of left auricular endocardium. Photographed with Wratten B filter (dark green). Note PAS-positive material in myocardial fibers (glycogen) and in center of necrotic endocardial lesion.

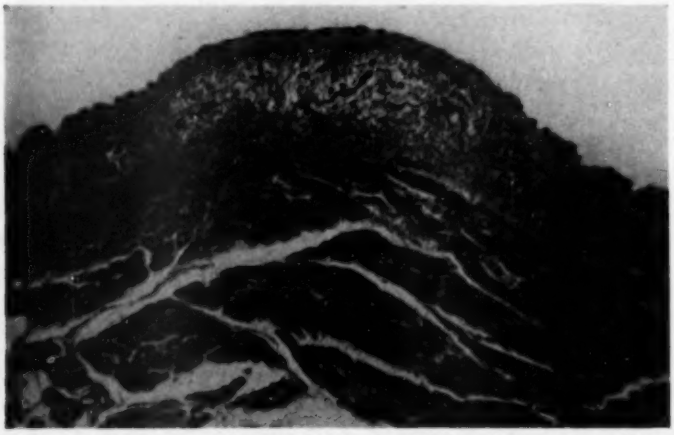

Fig. 4 (Dog P-6).—Hematoxylin and eosin stain of left auricular endocardium. Selected from group of supposedly "normal" dogs. Note focal area of thickened endocardium with swollen fragmented eosinophilic elastic fibers surrounded by clear areas. No leucocytic infiltration is present.

in Figure 4 with hematoxylin and eosin stain. This change consisted of small focal areas deep in the endocardium where there was separation of the fibers, swelling, and eosinophilia of collagenous and elastic fibers with occasional fragmentation but no cellular exudate or frank necrosis. The stains applied showed these clear areas to be occupied by Hale-positive (Fig. 5), metachromatic, PAS-negative (Fig. 6) material. Such deposits of varying size were found in 27% of the 22 "normal" dogs.

A small group of dogs which had been fed the high fat diet only for prolonged periods (37 to 77 weeks) were examined in a similar manner. None of these four dogs showed changes of either the necrotizing type or of the type found in normal dogs.

Fig. 5 (Dog P-6).—Hale stain of left auricular endocardium. Wratten F filter. Area corresponds with that shown in Figure 4, showing intense deposit of blue-staining material.

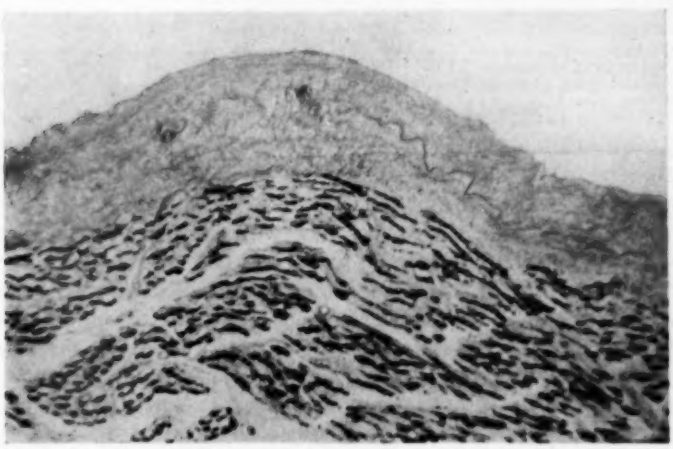

Fig. 6 (Dog P-6).—PAS stain of left auricular endocardium. Wratten B filter. Material which is demonstrated by Hale stain in Figure 5 is completely PAS-negative.

Another group of 16 dogs, fed only the regular kennel diet, were subjected to renal insufficiency either by injections of uranium nitrate or by bilateral nephrectomy. Only those that survived for five days or more and attained a blood urea nitrogen level of 150 mg. per 100 cc. or greater were included. Twenty-five per cent of these dogs showed deposits of mucopolysaccharide in the left auricular endocardium similar to those seen in the series of normal dogs.

In addition, a number of experimental dogs were studied which had been fed the high fat diet and subjected to renal insufficiency in the usual manner but in which the development of the necrotizing lesions had been prevented by various means, including corticotropin (ACTH) (two dogs), cortisone (two dogs), vitamin E (two dogs), cholesterol (four dogs), pregnancy (three dogs), adenosine-5-phosphate (two dogs), and vitamin mixtures (nine dogs).[9] These factors have been found previously to prevent the development of the typical arterial and endocardial lesions in a high percentage of dogs which would otherwise be expected to have them. Only 37% of this group showed any increase in deposition of acid mucopolysaccharide in the endocardium, and two of those with an increase had definite necrotizing lesions.

The results are summarized in the Table.

Certain quantitative features not brought out in the table are worthy of note. In the group of normal dogs and in the group subjected to renal insufficiency only, none of those classified as having a definite increase in acid mucopolysaccharide showed as much as 25% of the auricular endocardium contained in the section to

The Amount of Acid Mucopolysaccharide in the Left Auricular Endocardium of Dogs Subjected to Various Experimental Procedures

Experimental Procedure	Total Number Dogs	Dogs with Typical Necrotizing Lesions	Estimate of Increased AMPS * in Microscopic Sections			Per Cent with Increased AMPS *
			0	±	+	
Normal	22	0	16	0	6	27
Renal insufficiency only	16	0	12	0	4	25
High fat diet only	4	0	4	0	0	0
High fat diet plus renal insufficiency	11	7	2	0	9	82
High fat diet plus renal insufficiency plus protective factor †	24	2	14	1	9	37

* AMPS; acid mucopolysaccharide.
† See text.

be involved. Most of the deposits, in fact, were quite small and scattered. On the other hand, five of the nine dogs in the group with high fat diet and renal insufficiency and seven of the nine dogs in the group with high fat diet, renal insufficiency, and some preventive factor which had a definite increase in acid mucopolysaccharide showed 25% or more of the endocardium contained in the section to be involved; and, in many of them, the excess mucopolysaccharide was present throughout the entire endocardium contained in a single section. In other words, the quantitative differences between the first three groups and the last two groups in the table were much more striking than is implied by the percentage alone.

COMMENT

As is implied by the distribution of the figures in the Table, even in normal dogs there is a wide variation in the amount of acid mucopolysaccharide in the left auricular endocardium as revealed by these methods. With the larger deposits, there are associated changes in the enclosed elastic and collagenous fibers similar to that seen in the early stages of necrosis as judged in conventional stains—that is, eosinophilia and swelling. The amount of this acid mucopolysaccharide is not significantly affected either by renal insufficiency alone, or by high fat feeding alone, but when

these are combined, there is a very definite increase of it in certain localized areas of the auricular endocardium, and necrosis proceeds on to fragmentation and complete dissolution of the tissue structure. In the later stages, the acid mucopolysaccharide itself is removed and there remain only necrotic debris and leucocytes which are usually associated with an increase of PAS-positive material.

The biochemical mechanism responsible for these lesions in fat-fed uremic dogs has not been identified. The evidence cited here appears to indicate that the local injury which precipitates the lesions is primarily an injury to the ground substance, in response to which an excessive amount is produced. Just what produces the extra acid mucopolysaccharide is not apparent, as there is no significant proliferation of fibroblasts (generally considered the source of the ground substance) and no obvious change morphologically in the fibroblasts which are present. Altshuler and Angevine [2] remark that acid mucopolysaccharide is often increased in areas without any apparent cellular proliferation.

Whatever the mechanism, it appears that the altered (injured?) ground substance can no longer maintain the integrity of the fibers, and, as these swell and fragment, leucocytes are attracted. The appearance does not suggest a primary injury to the fibers, for alterations in them are not seen without associated excess acid mucopolysaccharide, and occasional small deposits of excess mucopolysaccharide are seen without any abnormality of the fibers.

Another possible interpretation of the alteration in the mucopolysaccharide is that it is undergoing depolymerization with swelling and an increase in volume. The Hale and toluidine blue O stains do not answer the question of excess local production versus depolymerization, as the physicochemical nature of these reactions have not been elucidated. Gersh ‡ interprets metachromasia as increasing with decreasing polymerization. However, Sylven and Malmgren [12] report that with pure hyaluronic acid metachromasia increases with particle size. Gomori [13] and Pearse,[14] in their respective discussions of metachromasia, indicate no definite conclusion concerning the relationship of degree of metachromasia to polymerization.

With respect to the PAS reaction, likewise, there is uncertainty. Gersh associates intensification of the PAS staining with depolymerization. Pearse [14] states that the greater the polymerization, the more intense the PAS reaction. Gomori [13] writes that the staining of C_4-linked polysaccharides will not be affected by polymerism but that the C_3-linked polysaccharides will stain more intensely with decreasing chain length. Hence the interpretation of the appearance of PAS-positive material in the full-blown necrotic lesions is not clear. Its resistance to salivary digestion indicates that it is not glycogen. Prolonged extraction with hot methanol and chloroform to remove phospholipids was not done.

The presence of varying amounts of acid mucopolysaccharide in normal dogs is difficult to interpret. No renal lesions were observed grossly in any of these, nor was any abnormality observed microscopically in those examined. Such deposits were not found in sections from the right auricle, nor were they nearly so numerous in additional sections taken from portions of the left auricle away from that portion above the posterior cusp of the mitral valve, the usual site of the lesions. These

‡ References 5 and 11.

deposits appear quite similar to those described by Taylor [15] in the human aorta, where he noted the constant accumulation of acid mucopolysaccharide in areas showing fragmentation of elastic fibers.

SUMMARY

The necrotizing endocardial lesions produced by a high fat diet and renal insufficiency are characterized in their early stages by excessive accumulations of acid mucopolysaccharides which disappear as necrosis progresses. No change is caused by renal insufficiency alone or high fat diet alone. Such deposits appear to be the first morphologically detectable change in the development of these lesions, and it is suggested that the injury to the fibers and the subsequent lesions are mediated by an effect on the ground substance manifested by either excessive production or depolymerization. A surprisingly high percentage of "normal" dogs in the control series showed focal deposits of acid mucopolysaccharides in the endocardium of the left auricle. No explanation has been offered for this unexpected finding.

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News and Comment

Second Teaching Institute on Pathology, Microbiology, Immunology, and Genetics.—The second in a series of Teaching Institutes sponsored by the Association of American Medical Colleges will focus on pathology, microbiology, immunology, and genetics. The first one, held at Atlantic City in October 1953, covered the areas of physiology, biochemistry, and pharmacology.

The objectives of the Institute are to provide an opportunity for medical educators to discuss important teaching problems, to review current experiments in medical education, to exchange philosophies and experiences, and to make any suggestions which might improve the effectiveness of medical teaching and the educational opportunities offered to medical students.

The 1954 Teaching Institute will be held October 10-15. The Coordinators of Cancer Teaching will cooperate and participate in this Teaching Institute, and they will hold their own meeting October 15-16. The annual meeting of the association will follow on October 17-20. All meetings will be at the French Lick Springs Hotel, French Lick, Indiana.

Attendance at the Teaching Institute will be by invitation only and will be limited to 120 participants. One teacher from each of the 95 medical schools in the United States, Canada, Puerto Rico, and the Philippines has been nominated by the Committee and invited by the Association of American Medical Colleges, and the total group of participants will represent a balance among the several disciplines and areas that will be explored at the Institute.

In advance of the Institute, medical school participants will collect background information and opinions from their colleagues and schools for the use of committees in planning topics for discussion. The Institute will be a working conference, with participants meeting in groups of 10 to 15 and discussion will be entirely informal.

The Committee on the 1954 Teaching Institute, which is responsible for its organization and planning, is as follows:

*Douglas H. Sprunt, Professor of Pathology, University of Tennessee, chairman

Robert A. Moore, Vice-chancellor, University of Pittsburgh, co-chairman

*Granville A. Bennett, Dean, University of Illinois College of Medicine

Israel Davidsohn, Professor of Pathology, Chicago Medical School

*Charles A. Evans, Professor of Microbiology, University of Washington School of Medicine

Colin M. MacLeod, Professor of Microbiology, New York University College of Medicine

James V. Neel, Associate Professor of Internal Medicine, and Associate Geneticist, Institute of Human Biology, University of Michigan Medical School

*Robert E. Stowell, Professor of Pathology and Oncology, University of Kansas School of Medicine

*Emory D. Warner, Professor of Pathology, State University of Iowa College of Medicine

Stanley E. Dorst, Dean, University of Cincinnati College of Medicine; President, Association of American Medical Colleges

George Packer Berry, Dean, Harvard Medical School; Chairman, A. Am. M. Coll. Committee on Teaching Institutes and Special Studies

John M. Stalnaker, Director of Studies, A. Am. M. Coll.; Secretary, A. Am. M. Coll. Committee on Teaching Institutes and Special Studies

*Members of Executive Committee

The four basic areas for discussion at the Institute, and the subcommittee which will be responsible for each area, are as follows:

The Objectives of Teaching

Granville A. Bennett, chairman

Walter J. Burdette, Associate Professor of Surgery, Louisiana State University School of Medicine

Israel Davidsohn, Professor of Pathology, Chicago Medical School

Sidney C. Madden, Professor of Pathology, University of California School of Medicine (Los Angeles)

Walter J. Nungester, Professor of Bacteriology, University of Michigan Medical School

Alwin M. Pappenheimer, Jr., Professor of Microbiology, New York University College of Medicine

Problems of the Teacher

Emory D. Warner, chairman

D. Murray Angevine, Professor of Pathology, University of Wisconsin Medical School

William C. Boyd, Professor of Immunochemistry, Boston University School of Medicine

Edwin A. Lawrence, Professor of Surgery, Indiana University School of Medicine

R. G. E. Murray, Professor of Bacteriology and Immunology, University of Western Ontario Faculty of Medicine

Interrelationships

Robert E. Stowell, chairman

James W. Moulder, Associate Professor of Biochemistry, University of Chicago School of Medicine

Ira T. Nathanson, Associate Clinical Professor of Surgery, Harvard Medical School

James V. Neel, Associate Professor of Internal Medicine, and Associate Geneticist, Institute of Human Biology, University of Michigan Medical School

Robert F. Parker, Associate Professor of Microbiology and of Medicine, Western Reserve University School of Medicine

James P. Tollman, Professor of Pathology and Bacteriology and Dean, University of Nebraska College of Medicine

Problems of the Student

Charles A. Evans, chairman

Thomas P. Almy, Associate Professor of Neoplastic Diseases (Medicine), Cornell University Medical College

John B. Graham, Assistant Professor of Pathology, University of North Carolina School of Medicine

William B. Wartman, Professor of Pathology, Northwestern University Medical School

Dennis W. Watson, Professor of Bacteriology and Immunology, University of Minnesota Medical School

Mrs. Helenor Campbell Wilder's Retirement.—Mrs. Helenor Campbell Wilder recently retired from her duties as Chief of the Section of Ophthalmic Pathology at the Armed Forces Institute of Pathology.

On May 2, 1953, she was honored as "Woman of the Year" for science by the Women's National Press Club. She is also to receive the Exceptional Meritorious Award, the highest civilian award in the Department of Defense.

Brigadier General Elbert DeCoursey, The Director of the Armed Forces Institute of Pathology stated that her work has advanced the study of the eyes more than 50 years. The disease of the eye which was demonstrated by Mrs. Wilder to be due to Toxoplasma had puzzled pathologists for generations.

Another eye disease found principally in children was also shown by Mrs. Wilder to be caused by migration of the larval stage of a worm. Mrs. Wilder's demonstration that blindness in these cases was due to infections rather than tumors enabled the attending physicians to institute appropriate treatment and indicated channels for future research.

Her abundant store of knowledge in the field of ophthalmic pathology has carried her to lecture from Baltimore to Tokyo. She is the first woman to be elected to honorary membership in the American Academy of Ophthalmology and Otolaryngology. Mrs. Wilder also holds membership in the American Association of Pathologists and Bacteriologists, which represents the only time in the history of that organization that a person was accepted without an M.D. degree.

Truly a credit to the Armed Forces Institute of Pathology and humanity in general, she will be missed.

Her replacement is Lieutenant Colonel Lorenz E. Zimmerman.

Dr. Martland Honored.—Dr. Harrison E. Martland has recently been honored by the city of Newark, N. J., in the dedication of its new $13,000,000 hospital as the Harrison E. Martland Medical Center. This was done in recognition of his 25 years as chief medical examiner of Essex County and 45 years as city pathologist. Dr. Martland is a past president of the New York Pathological Society, as well as former president of the New Jersey and Essex County Medical Societies. The annual Harrison E. Martland Medical Lecture was established by the New Jersey Pathological Society in 1939. Dr. Martland has contributed richly to pathology, both from his experience as a medical examiner, and in his early studies in connection with radium poisoning of the workers who applied luminous paint to watch dials. He also contributed importantly to the Manhattan Project in World War II.

Seminar.—A three-day seminar on Application of Histochemistry to Pathology has been scheduled to be given at the Armed Forces Institute of Pathology, May 3-5, 1954, inclusive, for a group of approximately 50 military and civilian pathologists.

The course consists of a basic and comprehensive survey of chemical and physical methods which can be used by the pathologist for the study of sections of tissue under the microscope. The subjects include a review of the theoretical basis of histochemical and classical staining methods, the practical histochemistry of groups of chemical compounds, such as carbohydrates, lipids, pigments, and enzymes, and the histochemistry of particular organs, such as the kidney and pituitary gland.

The material will be presented by lectures, laboratory demonstrations, and the study of microscopic slides.

Kolson Memorial Lecture.—Dr. Sidney Farber of Boston, delivered The Dr. Jack W. Kolson Memorial Lecture at Sinai Hospital, Baltimore, on March 25, 1954.

Course in Medical Mycology.—A course in medical mycology has been offered continuously during the summer for the past seven years at Duke University School of Medicine and Duke Hospital. The course will be offered again this summer and has been designed to give the student a working knowledge of the human pathogenic fungi and an understanding of the diseases which they cause.

Instruction by members of the departments of medicine, pathology, and bacteriology will emphasize the clinical, pathological, and therapeutic aspects of fungus infections. Patients, clinical materials, cultures, and laboratory animals will be available for study. An opportunity to study pathological materials, gross and microscopic, will be given those whose interest and previous training would make this of value to them. The practical laboratory aids which help to establish a definitive diagnosis will be stressed.

The course is open to clinicians, pathologists, bacteriologists, technicians, and others who have an interest in the medical phases of mycology. Classes meet six days a week from July 5 to July 31.

Inquiries concerning this course should be directed to Dr. Norman F. Conant, Duke Hospital, Durham, N. C.

Meetings.—The next International Congress of Clinical Pathology will be held Sept. 6-10, 1954, in Washington, D. C. There will be some concurrent and some joint meetings with the Fifth International Conference on Geographic Pathology and of the International Association of Medical Museums. In addition, the College of American Pathologists will sponsor a half-day symposium on the first day, and the American Society of Clinical Pathologists will conduct the regular seminar on the day after the Congress, Sept. 11.

Awards.—Dr. Peyton Rous received the Bertner Foundation Award on April 5, 1954, in connection with the Eighth Annual Symposium on Fundamental Cancer Research, conducted by The University of Texas M. D. Anderson Hospital and The Tumor Institute.

Wisconsin Society of Pathologists.—The following officers were elected by the Wisconsin Society of Pathologists at their recent annual meeting: Dr. Edward A. Birge, Milwaukee, president; Dr. Maxwell B. Llewellyn, Janesville, vice-president; Dr. Robert S. Haukohl, Milwaukee, secretary-treasurer.

Deaths.—Dr. John H. Mueller, Professor and Head of the Department of Bacteriology and Immunology at the Harvard Medical School, Boston, died in Boston, Feb. 16, 1954, at the age of 62.

Deaths.—Dr. James B. Herrick, Chicago, died on March 7, 1954, at the age of 92. His death occurred 42 years after his monumental contribution to the subject of coronary thrombosis.

Books

Surgical Pathology. By Peter A. Herbut, M.D.; Professor of Pathology, Jefferson Medical College, and Director of Clinical Laboratories, Jefferson Medical College Hospital, Philadelphia. Second Edition. Price, $14.00. Pp. 893, with 528 figures. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1954.

It is perhaps unfortunate that the term "surgical pathology" was ever created, suggesting as it does, that what the surgeon needs to know is one branch of pathology, and what the nonsurgeon should know is something else. Nevertheless, out of the present overwhelming array of facts and theories, the surgeon has to be more familiar with some aspects than with others. Hence a book of this type, which presents those data of pathology most useful to a surgeon. The selection of material which pleases one author or critic might seem superfluous to another or insufficient to still another. Dr. Herbut's book is of generous dimensions, with good bibliographies and over 500 illustrations. The quality of the illustrations is generally high, although a few are definitely of inferior grade. The book tends toward too encyclopedic coverage, which is inadequate for the specialist and too much for the beginner. It seems to this reviewer that the text might have dealt with some of the common conditions in much greater detail. For example, cystic hyperplasia of the breast is very briefly treated, covering three and one-fourth pages of text, including illustrations, while mesothelioma of the pleura covers almost two pages. For most of the regions of the body there exist more specialized texts of pathology, and a one-volume work cannot compete with the aggregate of all such special works. Nevertheless the book will be of great value to the surgeon, whether in practice or in training, while the pathologist can profitably draw on Dr. Herbut's accumulated experience.

Clinical Chemical Pathology. By C. H. Gray, D.Sc., M.D., M.R.C.P., M.R.C.S., F.R.I.C.; Professor of Chemical Pathology, University of London; Chemical Pathologist, King's College Hospital, London. Price, $3.00. Pp. 138, with 17 figures. Williams & Wilkins Co., Mount Royal and Guilford Aves., Baltimore 2, and Edward Arnold & Co., 41-43 Maddox St., London, W.1, 1953.

This small volume should prove one of the most useful publications of the year. In brief compass it presents the salient feature of certain biochemical aspects of disease. The major topics include renal function, acid base balance, fluid balance and edema, liver function, blood sugar, calcification, metabolism of fats, endocrine diseases, and others. Because of its compactness, it is not a book for the beginner. But for the person who has already been exposed to biochemistry, this volume presents an excellent compendium and summary. It is highly recommended for house officers, clinicians, laboratory workers, and pathologists who wish to have a good digest of modern biochemistry. It does not take the place of the larger and more detailed reference books.

1954 Medical Progress: A Review of Medical Advances During 1953. Edited by Morris Fishbein, M.D. Price, $5.00. Pp. 345. Blakiston Company, 575 Madison Ave., New York 22, 1954.

The Pathology of Trauma. By Alan R. Moritz, M.D., Professor of Pathology and Director of the Institute of Pathology of the School of Medicine of Western Reserve University, Cleveland. Second Edition. Price, $8.50. Pp. 414, with 126 illustrations. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1954.

The second edition of this standard work, now appearing 12 years after the first, is a very welcome addition to the pathologist's library. The text has been considerably enlarged and rewritten, and references brought up to date. The aid of eight collaborators is specifically acknowledged. Although the actual number of pages is only slightly increased, the format is altered to accommodate more text per page, so that the increased material is greater than

appears from mere pagination. Illustrations have been increased in number and remain of high quality. A new chapter on The Medicolegal Autopsy has been added. The extensive revisions include much information derived from war experience.

The physician, whether pathologist or clinician, who has contact with cases of trauma will find the book invaluable as an orientation and as a guide for interpretation of traumatic processes.

Klinik der Nebenniereninsuffizienz und ihre Grundlagen. By Prof. Dr. Ludwig Weissbecker, Medizinische Univ.-Klinik Freiburg/Br., with a foreword by Prof. Dr. Ludwig Heilmeyer, Director der Medizinischen Univ.-Klinik Freiburg/Br. Price, DM 31. Pp. 251. Ferdinand Enke, Hasenbergsteige 3, Stuttgart, W, 1954.

This monograph on adrenal deficiency replaces the older book by Thaddea on the same subject. It is a well-organized and thoroughly documented review of the subject beginning with the anatomy and histology of the glands and proceeding to such abstruse matters as the recognition and therapy of "sub-clinical" deficiency states. The writing is clear, and judgments are based, as much as possible, on the knowledge of physiology and biochemistry. The information is remarkably up-to-date, and the literature quoted is international in scope. The one general criticism this reviewer has to offer is that the desire to be all-inclusive and quote all papers makes the monograph a valuable source of reference, rather than a critical guide to the subject.

General Virology. By S. E. Luria, Professor of Bacteriology, University of Illinois, Urbana, Ill. Price, $8.50. Pp. 427, with 92 figures. John Wiley & Sons, Inc., 440 4th Ave., New York 16, 1953.

"General Virology" is a textbook designed for the student of the biological sciences who is primarily interested in the virus and its interaction with the host cell as a biological phenomenon which may illustrate and explain fundamental host-parasite relationships. It is not an applied medical virology reference for use of the clinician interested in the diagnosis and therapy of specific human viral diseases.

The author has chosen to view virology as a separate biological science in which the older fields of genetics, biochemistry, histology, and pathology play important parts in explaining the dual concepts of the virus particle and the virus-infected host cell. The virus is carried through its origins, its response to stimuli, and all of the phenomena attributable to its interactions. The vast potentialities of medical virology may be better realized after reading chapters on the impact of tissue culture on viral vaccine production, the virus-tumor relationship, and the oncolytic effect of viruses.

The basic knowledge of virology has been set down in this, the first practical textbook in a new science, in a highly readable and informative manner by a pioneer in the field whose personal investigations established much of our present knowledge of the field. The reader is left with an overall picture of this lusty new science that no other book has presented and clearly sees it in a frame of reference made up of the older sciences from which it has sprung.

The Hepatic Circulation and Portal Hypertension. By Charles G. Child, III, M.D. Price, $12.00. Pp. 444, with 132 illustrations. W. B. Saunders Company, 218 Washington Sq., Philadelphia 5; W. B. Saunders Company, Ltd., 7 Grape St., London, W.C. 2, 1954.

This is a scholarly and clearly written monograph which, although it restricts itself to the subject designated, should be of great interest to all concerned with pathological conditions involving the hepatic circulation. The early chapters thoroughly cover present knowledge of the comparative anatomy, embryology, gross and microscopic anatomy, and physiology of hepatic vasculature, both in normal and diseased states. There are chapters reviewing the role of the liver in shock and control of body water, and certain aspects of water, electrolyte, and protein balance in patients with cirrhosis. There is a rather complete consideration of the anatomy and physiology of the extrahepatic splanchnic circulation, particularly with regard to the effects of the Eck fistula and of hepatic artery ligation. In the concluding chapters the

problem of portal hypertension is discussed, particularly from the standpoint of surgical therapy, and the recent development of pancreatoduodenectomy with resection of the portal vein is presented.

This book considers each topic from the viewpoint of its historical development, citing major contributions, and the references form a valuable compilation of significant work in the field. The author draws heavily on his personal clinical experience in discussing portal hypertension and pancreatoduodenectomy. His experimental work on portal venography, hepatic regeneration in dogs after portal-caval transposition, the effect of epinephrine on portal venous pressure, and the effect of sudden occlusion of the portal vein, hepatic veins, and hepatic artery in monkeys is presented in detail. Surgical technique is not emphasized.

This represents a needed synthesis of a wealth of material from diverse sources, especially from basic work in anatomy and physiology, focusing on disease involving the portal venous system. In addition, its value lies in its foundation of the authors' own recent studies in this field.

Global Epidemiology: A Geography of Disease and Sanitation; Volume III. The Near and Middle East. By James S. Simmons, B.S., M.D., Ph.D., Dr.PH., Sc.D. (Hon.), Brigadier General, U.S.A., Retired; Dean and Professor of Public Health, Harvard University School of Public Health; Tom F. Whayne, A.B., M.D., M.P.H., Dr.P.H., Colonel, M.C., U.S.A.; Chief, Preventive Medicine Division, Office of the Surgeon General, U.S.A.; Gaylord W. Anderson, A.B., M.D., Dr. P.H., Mayo Professor and Director, School of Public Health, University of Minnesota; Harold M. Horack, B.S., M.D., Member of Staff, Department of Medicine and Section of Cardiology, Ochsner Clinic, New Orleans; Instructor in Medicine, Tulane University School of Medicine, and others. Price, $12.00. Pp. 357, with maps and tables. J. B. Lippincott Company, 227-231 S. 6th St., Philadelphia 5; Aldine House, 10-13 Bedford St., London W.C. 2; 2083 Guy St., Montreal, 1954.

Like its predecessors in the series on Global Epidemiology, Volume III, "The Near and Middle East" provides a systematic coverage of customs, climates and environmental circumstances which influence the health of the inhabitants of this section of the globe.

The Near and Middle East is really a vague geographical zone. The authors have considered this area to include Cyprus, Iraq, Israel, Hashemite Kingdom of the Jordan, Lebanon, Syria, Afghanistan, Iran, Turkey, and the Arabian Peninsula. Each nation is given an exhaustive report with regard to geography and climate, population and socioeconomic conditions, environment and sanitation, health services and medical facilities, and diseases. These broad headings, however, do not give a fair impression of the great wealth of detailed and valuable information packed into each section. In the discussion concerning Iran, for example, under the heading of "Environment and Sanitation" can be found the following: "Water Supplies," "Waste Disposal," "Fauna and Flora" (arthropods, reptiles, rodents, mollusks, and plants), and "Food Sanitation." In general, the coverage is thorough; however, on some of the nations considered only limited information is available. In discussing the diseases in Turkey the authors point out, "in general, the statistics are more reliable for the cities than for the smaller communities, many of which are situated at a considerable distance from established medical facilities. Moreover, about two-thirds of the inhabitants live in villages, where the deaths are certified by nonmedical officials, and inaccuracies in diagnosis are frequent." In other cases the information does not exist. For example in Yemen it is noted that certain respiratory diseases are encountered, "but no data are available regarding the prevalence of measles, whooping cough and other respiratory infections of childhood." These, however, are the exceptions to the rule; for the most part, much accurate data is available concerning each country. The basic information is reviewed in a valuable summary at the end of each chapter. The authors have drawn their material from a wealth of international literature and they supply a well-selected modern bibliography.

The style is direct, reading much like a public health report. There are 39 maps. Most of them are very simple and resemble rough tracings. Fourteen of these are epidemiological maps of limited areas. The four tables in the book are found in the appendix dealing with immunization procedures employed in tropical regions. The lack of tables and graphs in this book is

probably its only major fault. Much of the material could be presented in a composite and correlative manner through this means. The appendix carries the same admirable chapter, "Health Hints for the Tropics" which was printed in Volume II. There are a few changes in the tables, and a section on Mental Health in the Tropics has been added.

This volume, like the other two in the series, is an indispensable reference source for geographers, historians, and religious, diplomatic, industrial, public health, and military officials dealing with this section of the globe. It would, indeed, be useful in shaping an understanding of the background of these peoples and nations for anyone who follows with interest the news of international import which originates in the Near and Middle East.

Basic Pathology and Morbid Histology. By D. B. Cater, M.D., M.D. (Cambridge), F.R.C.S. (England), University Demonstrator in Pathology, University of Cambridge; Lector in Pathology, Trinity College, Cambridge; Formerly Head of the Surgical Unit, Lester Chinese Hospital, Shanghai. Price, $8.50. Pp. 330, with 263 figures. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1953.

In his preface the author explains that "this book is designed to help the student who has just began the study of Pathology and finds the subject difficult." To this end, the sonorous terms and stately tempo of the pathology textbook have been replaced by simple, familiar words whenever possible and by a casual, sprightly style which moves along very rapidly. Clinical features are used abundantly to emphasize the pathogenesis and course of disease, and analogies to military tactics, cricket, and other human activities and traits enliven the descriptions of cell behavior and tissue changes. There are no photographs, but numerous diagrams, charts, and greatly simplified microscopic drawings are included. The latter are somewhat disappointing, not so much in relation to the details omitted as in their inaccuracy; e. g., the depiction of Langhan's giant cells filled with large vividly eosinophilic granules.

It does not seem likely that this volume will suffice for medical students since it is neither a textbook in the usual sense nor a reference work. It may, however, find application in university courses for nurses, technologists, and others in ancillary medical fields.

Grenzen des Normalen und Anfänge des Pathologischen im Röntgenbilde des Skelettes. By Prof. Dr. A. Köhler. Ninth edition, revised by Dozent Dr. E. A. Zimmer, Bern/Fribourg. Price, DM 88. Pp. 672, with 1282 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, American agents for Georg Thieme, Diemershaldenstrasse 47, Stuttgart-O, 1953.

When Alban Köhler published the first edition of this book in 1910, he wanted to give the physician an atlas to recognize and interpret minor variations from the normal anatomic picture in the roentgenogram. It was to fill a gap between the presentation of the typical normal and the obvious pathologic. That Köhler succeeded in his task is well demonstrated by the fact that seven new editions appeared in about 30 years.

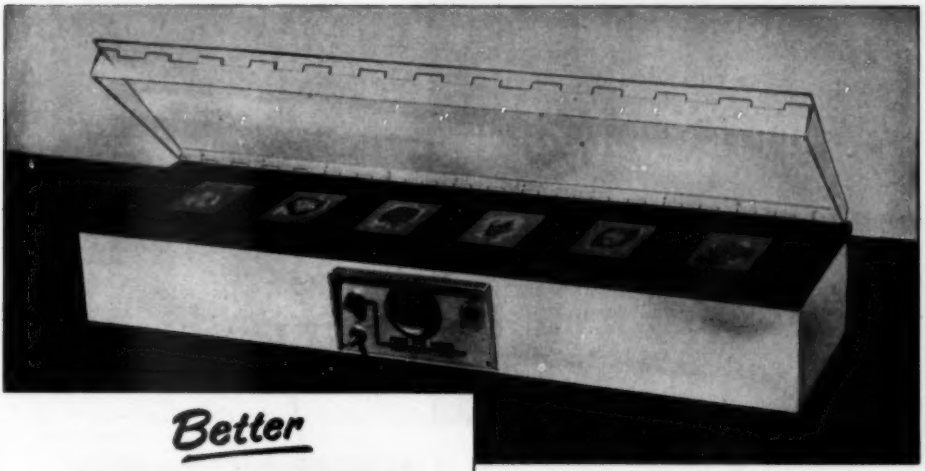
The present ninth edition was prepared by E. A. Zimmer and is actually a completely new book—retaining only the basic concept of the founder. More than 3,000 reports from the international roentgenologic surgical and orthopedic literature were consulted as a basis for the revision of the famous book and for the thorough bibliography.

Almost all the excellently reproduced figures are new, partly from the collections of the anatomic and pathologic institutes of Basel, Switzerland.

A relatively brief review covers the principles of the roentgenology of the bones, cartilages, joints, and soft tissues. Special emphasis is placed on sources of misinterpretation and mistakes. Then follows a chapter on the upper extremity, the shoulder girdle, thorax, skull, vertebrae, pelvis, and lower extremity.

Originally the text was planned as a reference book. The spirit of the book was best expressed by Köhler, who, from the wealth of his experience, stated that the roentgenogram alone is never sufficient for a diagnosis. Equally important are history and the clinical symptoms.

The book is valuable not only for the roentgenologist, it is also an excellent reference book for the pathologist interested in roentgenologic manifestations of borderline cases between the "normal" and the pathologic.



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